

Review

Plasmodial Kinase Inhibitors Targeting Malaria: Recent Developments

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Abstract: Recent progress in reducing malaria cases and ensuing deaths is threatened by factors like mutations that induce resistance to artemisinin derivatives. Multiple drugs are currently in clinical trials for malaria treatment, including some with novel mechanisms of action. One of these, MMV390048, is a plasmodial kinase inhibitor. This review lists the recently developed molecules which target plasmodial kinases. A systematic review of the literature was performed using CAPLUS and MEDLINE databases from 2005 to 2020. It covers a total of 60 articles and describes about one hundred compounds targeting 22 plasmodial kinases. This work highlights the strong potential of compounds targeting plasmodial kinases for future drug therapies. However, the majority of the *Plasmodium* kinome remains to be explored.

Keywords: kinase inhibitor; *Plasmodium*; malaria; SAR; medicinal chemistry

1. Introduction

Malaria is the deadliest parasitic disease with an estimated 405,000 deaths and 213 million cases in 2018 [1], mainly in Africa. Of the estimated victims, 67% are children below five years old. The disease is caused by five *Plasmodium* species: *P. falciparum* (responsible for most of the deaths), *P. vivax* and three less important species, *P. ovale*, *P. malariae* and *P. knowlesi*. With the use of artemisinin-based combined therapies (ACT), combining an artemisinin derivate and another antimalarial drug, and vector control measures, the World Health Organization hoped that 2020 would see a 40% reduction in malaria cases and deaths compared to 2015 [2]. However, this objective is not going to be achieved.

The vector, mosquitoes from the *Anopheles* genus, is becoming resistant to pyrethroid insecticides (used in long-lasting insecticide nets) in Africa [3]. Moreover, mutations on the *Pfkelch13* protein, plasmodial protein recently described to be involved in hemoglobin endocytosis mechanisms [4], mediate resistance to artemisinin and its derivatives. *Pfkelch13* mutations are linked [5]:

- In vitro to a decreased susceptibility during a ring stage assay,
- In vivo to a delayed parasitic clearance.

Increased prevalence of these mutations in South-East Asia is leading to clinical failures of ACT treatments [6]. Millions of people in Africa are threatened: recent researches indicate the appearance of in vitro artemisinin resistance in Rwanda [7], and the spread of city-dwelling *A. stephensi* in Africa [8]. Thus, research and development aimed at finding new drug therapies with novel mechanisms of action is a priority. Medicines for Malaria Venture, a non-profit organization, aims to develop new antimalarial drug therapies. To guide drug discovery, target candidate profiles (TCP) have been defined for antimalarial molecules setting clear goals for new therapy [9]. There are five TCP: blood-stage killer, hypnozoites killer, hepatic schizonts killer (chemoprotection), gametocytes killer, and transmission

blocker. The best compounds are the one following multiple or every TCP. One of these drug candidates, MMV390048, currently in phase II clinical trial, is the first plasmodial kinase inhibitor to reach this stage, opening the way for antimalarial drugs targeting this type of protein.

Kinases are proteins catalyzing the addition of a phosphate group on a substrate, like a simple molecule or a protein. In the latter, the kinase, designed under the term of “protein kinase”, will modified the activity of the targeted protein after addition of the phosphate group. The complete set of encoded protein kinases in the genome of an organism is defined as a kinome. The human kinome consists of 518 encoded kinases [10] that play a role in the regulation of cellular processes, and whose dysregulation is involved in numerous cancers [11]. The two first human kinase inhibitors approved by the Food and Drug Administration (FDA) were rapamycin in 1999 (indicated for immunosuppression) and imatinib in 2001. Importantly, the small molecule imatinib inhibits the BCR-Abl chimeric protein, found mainly in chronic myeloid leukemia. This finding accelerated research on human kinase inhibitors and as of 1 March 2019, 48 small molecules designed as human kinase inhibitors were approved by the FDA [12], the majority of them used in oncology. According to PKIDB (a website tracking human kinase inhibitors in clinical trials), as of July 2020, 253 human kinase inhibitors were currently in clinical trials [13].

Mapping of the *P. falciparum* kinome, depending on the literature source, indicates 85 (65 for eukaryotic protein kinases (ePKs) and 20 for ePKs-related proteins) [14] or 99 [15] encoding genes, and a significant number of these proteins do not possess a human ortholog [16]. These kinases are highly conserved among the different *Plasmodium* species. Moreover, 36 plasmodial kinases (in *P. falciparum*) are identified as likely to be essential for the asexual blood-stage [17], and 15 kinases are known to be involved in *P. berghei* development in mosquitoes [18]. Thus, developing selective plasmodial kinase inhibitors could lead to multiple new antimalarial drugs with new mechanisms of action.

This review of the literature lists molecules proven to be inhibitors of plasmodial kinases and describes their properties.

2. Methods

This systematic review complies with the PRISMA statement defining the PRISMA checklist and PRISMA flowchart [19].

2.1. Data Sources and Search Parameters

Two databases were used: Scifinder (<http://scifinder.cas.org>, CAPLUS and MEDLINE database) and Pubmed (<https://pubmed.ncbi.nlm.nih.gov/>, MEDLINE database). In both databases, the keywords and Boolean operator “kinase inhibitor AND malaria” were used. The advanced search options were set to “journal” for document type and English or French for language. The research was conducted on articles published from 1 January 2005 to 1 August 2020.

2.2. Article Selection

Articles were selected by one reviewer, with two rounds of selection. During the first round, for each database set, articles’ titles and abstracts were assessed to eliminate irrelevant material (outside medicinal chemistry or biological fields, non-malaria related, non-filtered reviews, duplicates). To be discussed in the following review, articles had to follow the inclusion and exclusion criteria listed in Table 1.

Table 1. Inclusion and exclusion criteria used.

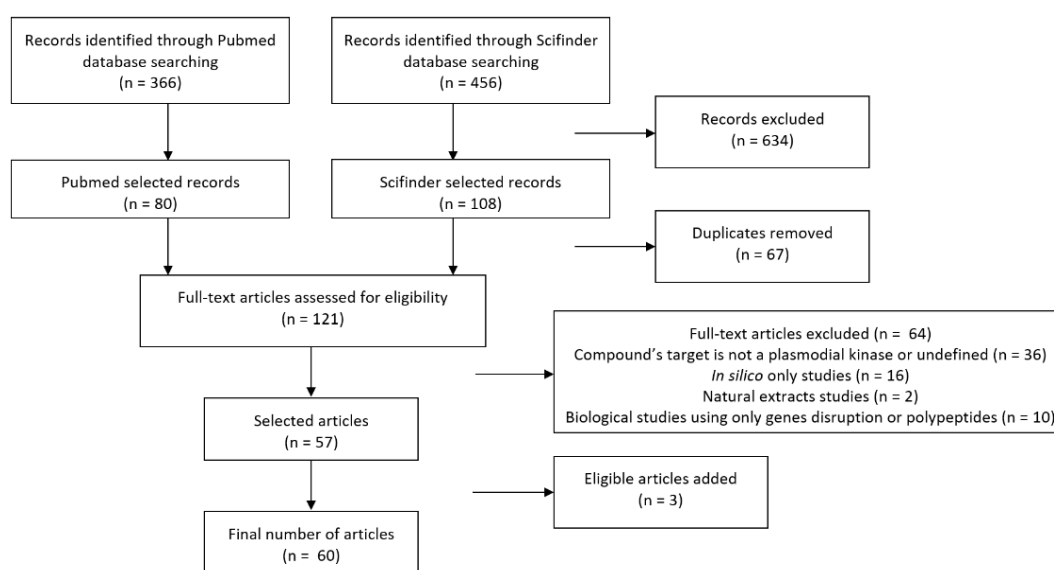
Parameter	Inclusion	Exclusion
Compound's target	A plasmodial kinase clearly defined as target	Target is not a plasmodial kinase or target is undefined
Study type	Medicinal chemistry studies Biology studies listing properties of one or more chemical compounds	In silico studies without biological tests Natural extract studies without active compounds determined Biological studies on protein pathways/phenotypic changes using only genes disruption or polypeptides

3. Results

Four hundred and fifty-six articles from Scifinder (after applying the “remove duplicates” tool) and 366 articles from Pubmed were obtained. During the first round of selection, 348 Scifinder articles and 286 Pubmed articles were excluded.

The two sets were combined, and duplicates removed, leaving 121 articles. During the second round of selection, 64 articles were excluded leading to 57 selected articles; three additional articles were added in the dataset while searching for further protein information.

This process is summarized in Scheme 1 in accordance with the PRISMA flow diagram [19].



Scheme 1. Flow diagram of article selection (derived from PRISMA flow diagram [19]).

4. Discussion

4.1. Molecules Targeting *P. falciparum* Calcium-Dependant Protein Kinases (PfCDPKs)

CDPKs can be found in plants and alveolate protists including *Apicomplexan* parasites like *T. gondii* and *Plasmodium spp* [20]. They belong to the calmodulin-dependent kinases (CaMK) family [14]. The CDPK proteins, possessing seven members (CDPK1 to CDPK7), are involved in multiple parasitic stages [21], and constitute interesting targets as they are not found in humans. Only inhibitors for *PfCDPK1* and *PfCDPK4* have been described in the literature.

4.1.1. Molecules Targeting *P. falciparum* Calcium-Dependent Protein Kinase 1 (PfCDPK1)

As discovered by Zhao et al. [22], *PfCDPK1* is involved in the invasion of red blood cells (RBC) by merozoites and their egress through activation of a protein motor complex [23,24]. More recent studies

indicate the role of *Pf*CDPK1 in gametogenesis, mosquito infection [25], and regulation of *Pf*PKA (*P. falciparum* (cAMP)-dependent protein kinase) [26]. *Pf*CDPK1 was assumed to be an essential protein for *Plasmodium* since knocking out the gene during blood-stages was impossible [24]. Bansal et al., starting from a *P. falciparum* strain with a mutant *Pf*CDPK1 having reduced activity, recently managed to knock out completely *Pf*CDPK1 [25].

In 2008, Green et al. [23] used compound K252a (Figure 1, compound 1) to study the motor complex regulated by *Pf*CDPK1. This indolopyrrolocarbazole displayed an IC_{50} of 45 nM toward *Pf*CDPK1 and was able to reduce RBC invasion by merozoites with an EC_{50} of 348 nM.

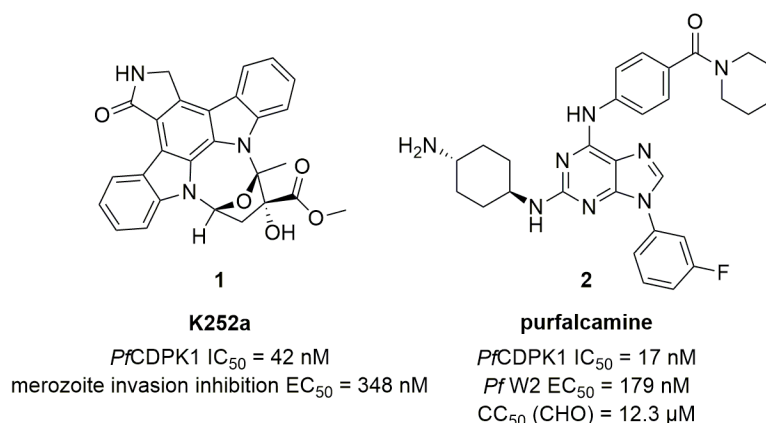


Figure 1. Inhibitors of *Pf*CDPK1 K252a 1 and purfalcamine 2.

Kato et al. screened a library of 20,000 compounds on *Pf*CDPK1 [24]. The most active class of compounds was a series of 2,6,9-trisubstituted purines and the most potent molecule, called purfalcamine (Figure 1, compound 2), showed an IC_{50} value of 17 nM on *Pf*CDPK1. EC_{50} in a parasitic proliferation assay on multiple *P. falciparum* strains including a multi-resistant W2 strain was determined, as well as cytotoxic values on multiple cell lines. However, the compound lacked activity in vivo in the four-day Peter's test [27]: only a delay in onset of parasitemia was observed. Other compounds from the 2,6,9-trisubstituted purine series lacked *Pf*CDPK1 affinity or *P. falciparum* in vitro activity when the fluorine atom was replaced by a carboxylic acid or a *tert*-butoxy group. *P. falciparum* in vitro activity was also suppressed when the 4-aminocyclohexyl was replaced by a 4-hydroxycyclohexyl group.

In 2009, Lemerrier et al. identified two *Pf*CDPK1 inhibitors after a screening of 54,000 compounds [28]: the indolizine 3 and the imidazopyridazine 4 (Figure 2). 3 displayed a K_i of 262 nM on *Pf*CDPK1, while 4 possessed a K_i of 37 nM on *Pf*CDPK1 and an IC_{50} on *P. falciparum* of 5.7 μ M. Out of 46 human kinases tested, compound 3 inhibited two human kinases and compound 4, five (at 10 μ M concentration). Modulation work focusing on the phenolic hydroxyl group of 4 showed a tolerance on the K_i value when this 3-hydroxyl was replaced with H-bond acceptors like 3,4-methylenedioxy or 3-methoxy, but in the latter accompanied with a 4-hydroxyl substitution.

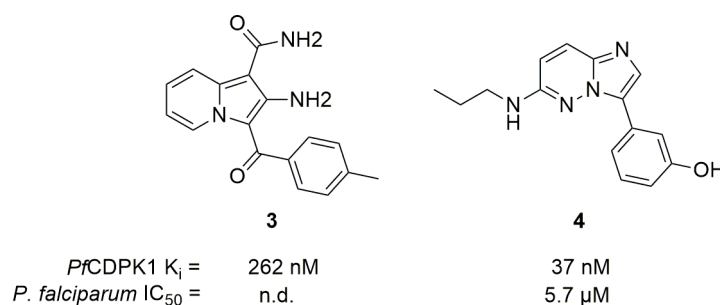


Figure 2. Inhibitors of *Pf*CDPK1 discovered by Lemerrier et al. [28].

Screening of 35,000 compounds by Chapman et al. [29] displayed imidazopyridazine as a promising series, in line with the discovery of compound 4. An extensive Structure-Activity Relationship (SAR) work started on this scaffold (Table 2). Chapman et al. [30] explored the side chains at positions 3 and 6 of the imidazopyridazine core. Work on the R₁ substituent largely targeted the introduction of cyclic amines, while work on R₂ addressed *para*- or *meta*-functionalized phenyls, pyridines, or pyrimidines. Compound 5 (Table 2) displayed interesting results with an IC₅₀ on PfCDPK1 of 13 nM, an IC₅₀ on Pf3D7 of 400 nM, and the ability to reduce by 46% the parasitemia, in a *P. berghei* mouse model *per os* during a four-day Peter's test. Finally, 5 showed inhibition toward 12 human kinases (out of 73 tested) ranging between 50% and 80% at 1 μM.

Table 2. Imidazopyridazine inhibitors of PfCDPK1.

Compound	R ₁	R ₂	PfCDPK1 IC ₅₀ (nM)	Pf3D7 IC ₅₀ (nM)	In Vivo Parasitemia Reduction ¹
5			13	400	46
6			70	103	-
7			<10	12	4%
8			12	80	51%

¹ Four-day Peter's test [27] at an oral dose of 50 mg/kg/day.

Large et al. continued this work with the introduction of *N*-substituted imidazoles at position 3 of the imidazopyridazine core while modulating position 6 with substituents like aminocyclohexyl or *N*-methylpiperidine [31]. This resulted in the synthesis of compound 6 (Table 2) showing an improved activity on Pf3D7 compared to compound 5 and improved ligand-lipophilicity efficiency (LLE) with a score of 6, compared to 4.5 for compound 5. Inhibition assays on human kinases were not performed on compound 6, but other compounds from this series showed lower selectivity than compound 5.

The last work on this series was done by Chapman et al. [32] using docking studies on the *T. gondii* CDPK1 ortholog structure to guide them. Starting from 5, they started by modulating R₂ with *N*-substituted 2-aminopyrimidines, hoping for improved in vivo results. Compound 7 (Table 2) emerged as the best compound with improved in vitro parameters. However, low PAMPA (parallel

artificial membrane permeability assay) permeability ($4 P_{app}/nms^{-1}$) led to poor results in vivo, with only 4% reduction of parasitemia in the four-day Peter's test [27]. Out of 66 human kinases tested at 1 μ M concentration, 7 inhibited nine human kinases by at least 80%. Additional work was then done: the side chain at position 6 was modulated with different cyclic amines, and at position 3, the fluorine atom position on both cycles was explored along with the nature of the two cycles (phenyls or pyridines). This led to compound **8** (Table 2) with slightly lower activity in vitro than **7**. The reduction of parasitaemia in *P. berghei* mouse model was slightly better at 51% than that of **5** at 46% (Table 2).

In 2016, Crowther et al. screened 14,000 compounds on multiple plasmodial kinases including *Pf*CDPK1 [33]. One hundred and eighty-one molecules showed a sub-micromolar IC_{50} on *Pf*CDPK1 and 12 compounds grouped in four chemical series displayed an IC_{50} equal to or below 20 nM. The best compounds from each series are represented in Figure 3.

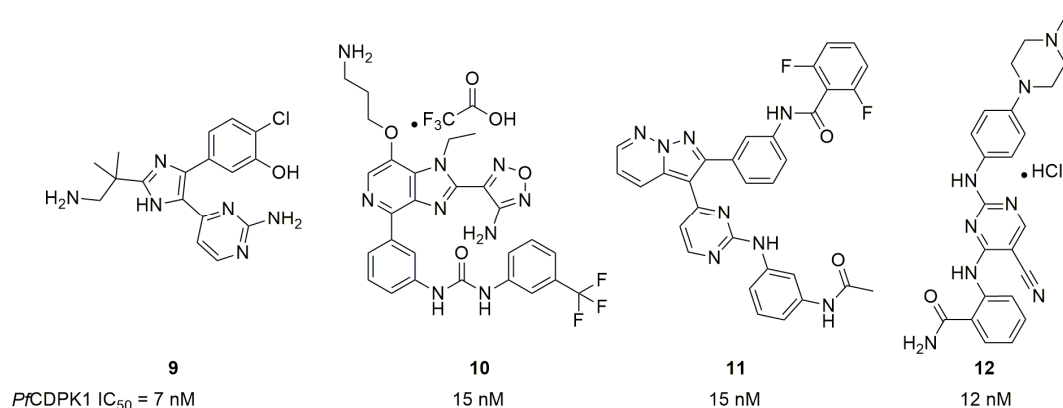


Figure 3. Best compounds, from each chemical series having a molecule with an IC_{50} equal to or below 20 nM toward *Pf*CDPK1 in the screening realized by Crowther et al. [33].

4.1.2. Molecules Targeting *Plasmodium falciparum* Calcium-Dependent Protein Kinase 4 (*Pf*CDPK4)

*Pf*CDPK4 is a key enzyme for the exflagellation of gametocytes, a mechanism leading to the formation of microgametocytes, the male gametocytes, in the mosquito midgut [34–36].

In 2012, Ojo et al. described the properties of BKI-1 (compound **13**), a disubstituted pyrazolo[3,4-*d*]pyrimidin-4-amine [36] (Figure 4). **13** displayed an IC_{50} on *Pf*CDPK4 of 4.1 nM, an IC_{50} on *Pf*CDPK1 of 136 nM, an EC_{50} toward asexual stages of 2 μ M (unsurprisingly since CDPK4 is important in the sexual stage) and was able to inhibit *P. falciparum* exflagellation with an EC_{50} of 35 nM. **13**'s selectivity was assessed with IC_{50} measures on *Hs*ABL and *Hs*SRC, two human kinases, who were superior to 50 and 20 μ M, respectively. Lastly, on a *P. berghei*-infected mouse model, **13** was able to completely inhibit the formation of oocysts in mosquitoes at the intraperitoneal dose of 10 mg/kg.

Ojo et al. then carried out minor pharmacomodulations on **13** [37] including the replacement of the piperidine with a *N*-methylpiperidine, morpholine or pyrane heterocycle, replacement of the naphthalene by a quinoline and replacement of the methoxy group by an ethoxy group. Activities on the enzyme and the parasite were maintained for all molecules except the quinoline analog without a methoxy group. Compound **14**, the *N*-methylpiperidine analog of **13** (Figure 4) showed similar in vitro activity parameters to **13** but clear improvements in in vitro and in vivo absorption-distribution-metabolism-elimination-toxicology (ADMET) parameters (Table 3). Selectivity against human kinases was conserved as only one protein out of the 80 assessed was found to be inhibited. The only downside to **14** was its hERG channel activity, which was reduced, from 0.767 μ M to more than 10 μ M, by replacing the *N*-methylpiperidine with a pyrane.

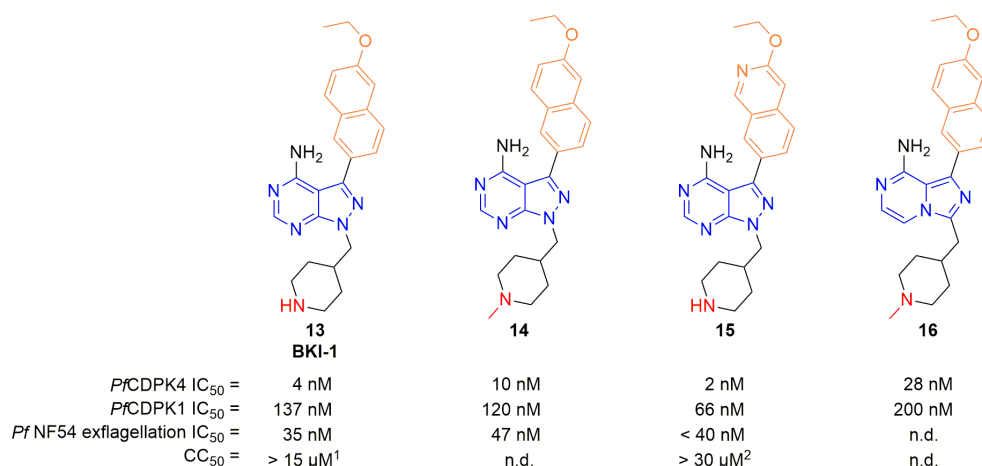


Figure 4. 4-Aminopyrazolopyrimidine and 4-aminoimidazopyrazine inhibitors of *Pf*CDPK4.

¹ On mammalian fibroblast cells. ² On HepG2 cells.

Table 3. ADMET parameters of compound **13** and **14** [37].

Compound	In Vitro			In Vivo	
	Solubility (μM) in Buffer pH 6.5	t _{1/2} (min) Human Liver Microsome	AUC ¹ (μM·min) at 10 mg/kg per os	AUC ¹ (μM·min) at 10 mg/kg Intraperitoneal	t _{1/2} (h) at 100 mg/kg per os
13	47	-	57	317	-
14	82	>60	430	863	13.5

¹ AUC = Area Under Curve.

This work was followed by a massive medicinal chemistry study by Vidadala et al. focused on the modulation of the lateral chains of the pyrazolo[3,4-*d*]pyrimidin-4-amine core [38]. ATP binding sites of both *Pf*CDPK1 and *Pf*CDPK4 are very similar to the one found in *T. gondii* CDPK1. Thus *Tg*CDPK1 was used for docking studies with previously described *Pf*CDPK4 inhibitors to guide pharmacomodulations. The C₃ side-chain was the first to be modulated while keeping an isopropyl or *tert*-butyl group as *N*-substituent. The best C₃ group was then used during the modulation of the *N*-substituent. A scaffold hopping strategy was also carried out, changing the pyrazolopyrimidine to an imidazopyrazine core. This led to compound **15** with an interesting dual activity on *Pf*CDPK1 and *Pf*CDPK4 (Figure 4) and a conserved selectivity versus *Hs*SRC (IC₅₀ > 10 μM). The scaffold hopping strategy was successful, as illustrated by compound **16**. The authors stated that they are now seeking to enhance the metabolic stability of their compounds in vivo, since they need to remain in the bloodstream for a period of three to four weeks to have their effect on gametocytes.

In 2016, Huang et al. used on 5-aminopyrazole-4-carboxamides, known to work on *Toxoplasma gondii* and *Cryptosporidium parvum* CDPK1 (orthologs of *Pf*CDPK4), to obtain new *Pf*CDPK4 inhibitors [39]. They synthesized 28 compounds; most of which have IC₅₀ toward *Pf*CDPK4 below 100 nM. The best compound regarding *Pf*CDPK4 inhibition was **17**, while **18** was interesting with a dual *Pf*CDPK1/4 inhibition (Figure 5). These two compounds did not possess any activity on hERG channels. **17** was selective as it possessed an IC₅₀ superior to 30 μM against *Hs*SRC. Work on this series is currently focused on *C. parvum* therapy [40].

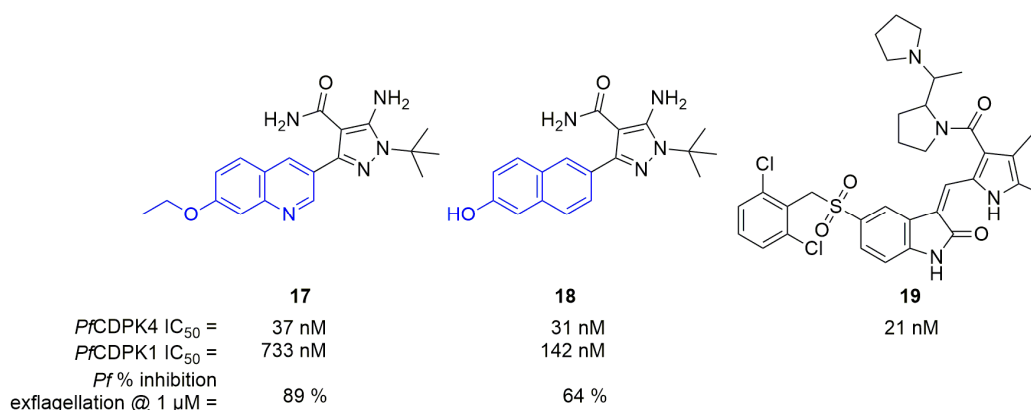


Figure 5. Aminopyrazole-carboxamide and indolone inhibitors of *Pf*CDPK4.

In the Crowther et al. screening [33], 55 compounds showed an activity below the micromolar range against *Pf*CDPK4. The best compound (**19**) displayed an IC₅₀ of 21 nM (Figure 5).

The only thing that prevents *Pf*CDPK4 inhibitors from entering more advanced pre-clinical studies is the long-term bioavailability they would need to be active against gametocytes. Moreover, finding from Vidadala et al. [38] and data from screening by Crowther et al. [33], identified compounds providing dual inhibition of *Pf*CDPK1 and *Pf*CDPK4, offering the prospect of compounds with action on two stages out of three of the *Plasmodium* cycle.

4.2. Molecules Targeting *Plasmodium falciparum* Choline Kinase (*Pf*CK)

Choline kinase is an enzyme involved in the synthesis of phosphatidylcholine; phospholipid most frequently found in *P. falciparum*. It transforms choline into phosphocholine, and is also involved in the synthesis of phosphatidylethanolamine, the second most common phospholipid [41]. Inhibition of *Pf*CK has been shown to reduce parasite growth in vitro [42,43]. The active sites of *Pf*CK have 69% similarity with the human enzyme *Hs*CKα1 [44].

In 2007, Choubey et al. [45] studied hexadecyltrimethylammonium bromide (compound **20**, Figure 6). It displayed in vitro, at 10 μM and 20 μM, parasitic growth inhibition of 62% and 81%, and reduced phosphocholine synthesis by 57% at 10 μM. In a *P. yoelii* mouse model, it was able to reduce parasitemia by almost 50% after four days of intravenous doses of 5 mg/kg.

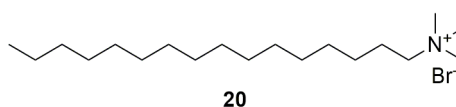


Figure 6. Hexadecyltrimethylammonium bromide **20** structure.

A screening by Crowther et al. on around 5000 commercially available small molecules led to the discovery of three hits against *Pf*CK [46] (compounds **21**, **22** and **23**, Figure 7).

While studying *Pf*CK metabolic activities, Serrán-Aguilera et al. used two compounds (**24** and **25**, Table 4) to inhibit the enzyme [41], which possessed a K_i between 30 and 40 μM (when assessed for choline conversion to phosphocholine). To explore the activity of these pyridinium salts on *Pf*CK, Schiafino-Ortega et al. assessed 1,2-diphenoxyethane salts with a symmetrical structure [47] close to **24** and **25**. Bisquinolinium bromide salts derivatives were the most potent with submicromolar activity on *P. falciparum*. However, their activity on the *Pf*CK enzyme was found to be above 2 μM for all this series, raising doubts about their real mechanism of action. The best compound of this series was compound **26**, with an improved *Pf*CK_{Cho} IC₅₀ (formation of phosphocholine is measured) reduced from 103 to 2.4 μM, but it no longer showed *P. falciparum* in vitro activity (Table 4). Compound **26** was not selective as it also inhibited the human *Hs*CKα1 enzyme.

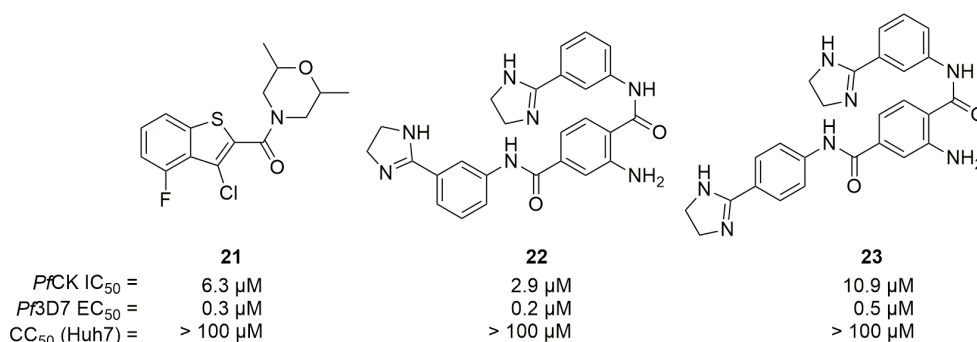


Figure 7. Hits from Crowther et al. against *PfCK* [46].

Table 4. Bispyridinium bromide salt and bisquinolinium bromide salt inhibitors of *PfCK*.

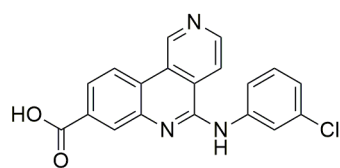
Compound	<i>PfCK</i> _{Cho} IC ₅₀ (μM)	<i>Pf3D7</i> IC ₅₀ (nM)	<i>HsCKα1</i> IC ₅₀
<p style="text-align: center;">24</p>	276	3	-
<p style="text-align: center;">25</p>	103	3	-
<p style="text-align: center;">26</p>	2.4	142	1.7

Most of the molecules presented above (from **21** to **25**) probably target more than just *PfCK*, as enzyme IC₅₀ values not in line IC₅₀ on the parasite: **24** is the best example of such behavior. Furthermore, the design of these compounds needs to take into account the human choline kinase. Thus, *PfCK* inhibitors are potentially valuable compounds, they are still in the early stages of development and promising hits have yet to be discovered.

4.3. Molecules Targeting *Plasmodium falciparum* Casein Kinase 2 (*PfCK2*)

Casein kinases are serine/threonine protein kinases found in eukaryotic organisms. Two casein kinases can be found in *P. falciparum*: CK1 and CK2. *PfCK2* is thought to be a key enzyme during the asexual blood-stage of the parasite. It has been shown that many proteins are possibly phosphorylated, and thus have their activity regulated, via *PfCK2* during the asexual blood-stage [48]. Both *PfCK1* and *PfCK2* have been demonstrated to be essential for the asexual blood-stage [17,49].

While studying the *PfCK2* catalytic domain, Ruiz-Carillo et al. used CX-4945 (Figure 8, compound **27**) [50], a human CK2 inhibitor currently in multiple phase II clinical trials in oncology [51], to inhibit *PfCK2* with an IC₅₀ of 13.2 nM.



27
CX-4945

*Pf*CK2 IC₅₀ = 13.2 nM

*Hs*CK2 IC₅₀ = 4.7 nM

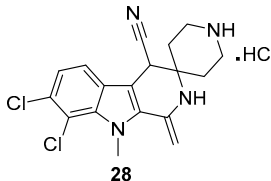
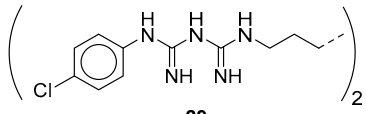
Figure 8. CX-4945, an *Hs*CK2 inhibitor in clinical trials, also inhibits *Pf*CK2.

4.4. Molecules Targeting *Plasmodium falciparum* Cyclin-Dependent-Like Kinase (*Pf*CLK)

The CLK family includes four enzymes (*Pf*CLK1 to *Pf*CLK 4) involved in the phosphorylation (and the activity modulation) mainly of serine-arginine-rich proteins found in spliceosomes [14,52,53]. Spliceosomes are complexes of proteins involved in the removal of introns in pre-messenger RNA. All *Pf*CLKs are considered essential for the asexual blood-stage [17]. Inhibition of CLKs was first linked to a schizonticide and gametocytocidal effect [53]. A more recent study on *Pf*CLK3 showed that inhibition of this protein affected all three stages of the malaria cycle [54].

In 2014, Kern et al. studied the effect of inhibiting *Pf*CLKs [53]. The authors did not manage to disrupt the genes directly and therefore turned to chemical compounds. They screened 63 *Hs*CLK inhibitors against *Pf*CLKs. Two compounds, **28** and chlorhexidine (**29**) showed interesting results regarding *Pf*CLK inhibition and *Pf*3D7 growth inhibition; results are summarized in Table 5.

Table 5. Summary of in vitro properties of *Pf*CLK inhibitors from Kern et al. [53].

Compound	<i>Pf</i> 3D7 IC ₅₀ (μM)	EIA ¹ IC ₅₀ (μM)	% Inhibition (at <i>Pf</i> 3D7 IC ₅₀ Concentration)			
			<i>Pf</i> CLK1	<i>Pf</i> CLK2	<i>Pf</i> CLK3	<i>Pf</i> CLK4
 <p>28</p>	4.4	13.8	73%	45.6%	35.1%	71.6%
 <p>29 Chlorhexidine</p>	0.6	19.8	68.4%	76.4%	74.2%	76.1%

¹ Exflagellation Inhibitory Assay.

In 2017, Bendjeddou et al. tested an imidazopyridazine series on multiple human and parasitic kinases, including *Pf*CLK1 [55]. Two compounds (**30** and **31**, Figure 9) showed activity on *Pf*CLK1 below 100 nM but lacked selectivity as they also targeted human kinases, in some cases with IC₅₀ below 100 nM.

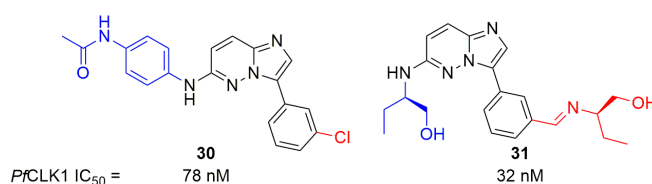


Figure 9. Imidazopyridazines inhibitors of *Pf*CLK1.

More recently, Alam et al. screened 30,000 compounds on *Pf*CLK1 and *Pf*CLK3 [54]. TCMDC-135793 (compound **32**) and TCMDC-135051 (compound **33**) were among the most potent and selective compounds on *Pf*CLK1 and *Pf*CLK3, respectively (Figure 10). Target was confirmed for **33** with *Pf*Dd2 strain possessing a mutant *Pf*CLK3 that showed an increased IC_{50} value. Compound **33** was active at all stages, inhibiting:

- Schizont development in blood-stage,
- Sporozoite invasion and development in the liver stage,
- Gametocyte development,
- Exflagellation in the mosquito midgut.

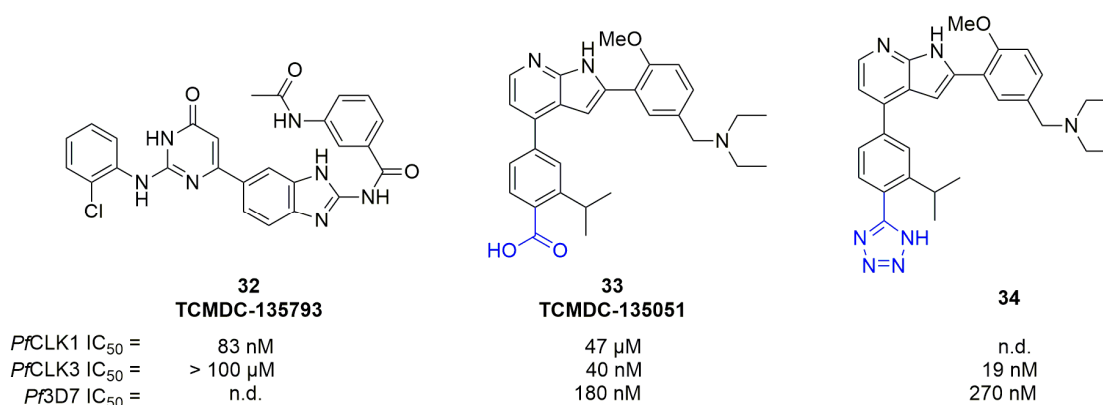


Figure 10. Molecules inhibiting *Pf*CLK1 or *Pf*CLK3.

SAR work associated with **33** was carried out by Mahindra et al. [56]. Modulations targeting the substituents of the phenyl ring at position 2 of the pyrrolo[2,3-b]pyridine core led to analogs possessing an IC_{50} against *Pf*CLK3 below 100 nM. However, micromolar activity was found against *Pf*3D7 when the diethylamine was changed to dimethylamine, primary amine or morpholine substituents. The position of the methoxy substituent appeared to be important for activity. Similar modifications were carried out on the other phenyl ring: removing the isopropyl substituent or replacing it by methyl led to compounds with micromolar activities against *Pf*3D7, and removing the carboxylic acid or changing it to an ethyl ester resulted in compounds with micromolar activity on *Pf*CLK3. Replacing the carboxylic acid to a tetrazole, a known bioisoster, led to compound **34** showing a slight improvement in *Pf*CLK3 activity, with an IC_{50} of 19 nM but with an increased *Pf*3D7 IC_{50} to 270 nM. **34** stability was conserved in vitro in mouse liver microsomes with an intrinsic clearance at 2.32 mL/min/g of liver, compared to **33** with 1.33.

4.5. Molecules Targeting Coenzyme A Synthesis Pathway Kinases: *Plasmodium falciparum* Pantothenate Kinase and Dephospho-Coenzyme A Kinase (*Pf*PanK & *Pf*DPCK)

Coenzyme A is a molecule found in many metabolic pathways in eukaryotic life forms. In *P. falciparum*, coenzyme A is synthesized in five steps starting from pantothenate; two of these steps involve kinases:

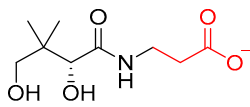
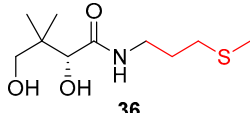
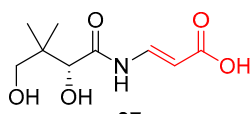
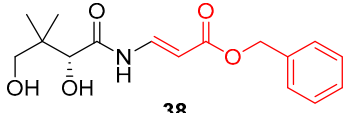
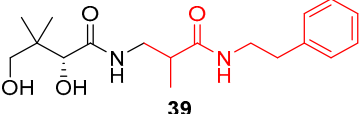
- the first step, transforming pantothenate into 4'-phosphopantothenate, is catalyzed by *Pf*PanK,
- the last step, transforming dephospho-coenzyme A into coenzyme A, is catalyzed by *Pf*DPCK.

While the synthesis and the function of coenzyme A are well preserved around all organisms that possess it [57], the enzymes' coding sequences can differ.

Studies on the inhibition of *Pf*PanK focused on the synthesis of pantothenate (Table 6, compound **35**) analogs able to competitively inhibit 4'-phosphopantothenate synthesis. Spry et al. synthesized ten

analogs with various chains on the nitrogen atom of the amide group [58]. Compound **36** was the best of the series, with an IC_{50} of 76 μ M on *Pf3D7* (at 1 μ M pantothenate concentration). Increasing the pantothenate concentration increased this IC_{50} suggesting a mechanism of action related to the pantothenate metabolism. Changing the terminal group of the chain or its length did not affect the activity. Spry et al. recently investigated the synthesis of analogs of CJ-15,801 (**37**) [59], a *trans* enamide analog of pantothenate. Modulations were focused on the ester and modifying the diol side chain. Compound **38** showed an improved IC_{50} of 13 μ M on *Pf3D7* compared to 36 μ M for **37** but most importantly a 100-fold decreased of the *PfPanK* IC_{50} value. In 2017, Chiu et al. described compound **39** as a pantothenate kinase inhibitor with an IC_{50} of 30 nM on *PfW2* [60]. Adding more pantothenate to the growing medium increased this value. The authors also demonstrated that **39** was able to inhibit the *PfPanK* homolog of *S. cerevisiae*, Cab1.

Table 6. Pantothenate and pantothenate analog inhibitors of *PfPanK*.

Compound	<i>P. falciparum</i> IC_{50} (μ M) ^{1,2}	Pantothenate Phosphorylation Inhibition %	<i>PfPanK</i> IC_{50}
 35 pantothenate	-	-	-
 36	76	>85% (at 1 mM)	-
 37	36	-	14 μ M
 38	13	>60% (at 10 μ M)	140 nM
 39	0.03	-	-

¹ *Pf3D7* strain except for **39**, where the value shown is on *PfW2* strain. ² Pantothenate concentration in growth medium was 1 μ M except for **39**, where it was 1.14 μ M.

Concerning *PfDPCK*, work by Fletcher et al. identified some potent compounds [61]. The activity of assessed compounds on *P. falciparum* was counterbalanced by adding coenzyme A to the culture medium to identified compounds active on coenzyme A synthesis. Similar rescue tests were then done with *PfPanK* or *PfDPCK* substrates. Four compounds (**40–43**, Figure 11) showed possible activity on *PfDPCK*, affecting the blood-stage and gametocytes.

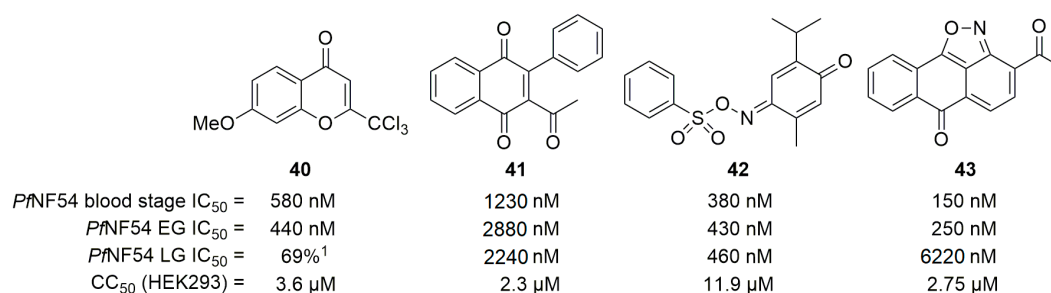


Figure 11. Inhibitors of *PfDPCCK* from Fletcher et al. [61]. ¹ At 40 μM. EG = Early-stage Gametocytes, LG = Late-stage Gametocytes.

4.6. Molecules Targeting *Plasmodium falciparum* FIKK Kinases (*PfFKks*)

FIKK kinases are named after a shared sequence of four amino acids (Phe-Ile-Lys-Lys or FIKK) and are an important group of kinases in malaria parasites [14]. While *P. vivax* and *P. berghei* possess one FIKK kinase, *P. falciparum* differs completely, possessing 20 FIKK kinases. The FIKK kinase gene may be essential for the parasite [18], yet the role of these proteins remains unclear. *PfFKks* are exported into the RBC cytosol (except *PfFKk8*), where they possibly interact with RBC's cytoskeletal proteins and membrane [62,63].

The only studies on inhibitors of FKks were performed by Lin et al. [64,65], who screened compounds on *PfFKk8* and *P. vivax* FKk (catalytic domain only for the latter). In both articles, emodin (**44**, Figure 12), an anthraquinone, showed an IC₅₀ of 1.9 μM and 2 μM against these tested proteins, respectively.

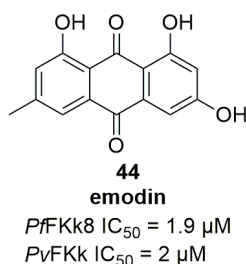
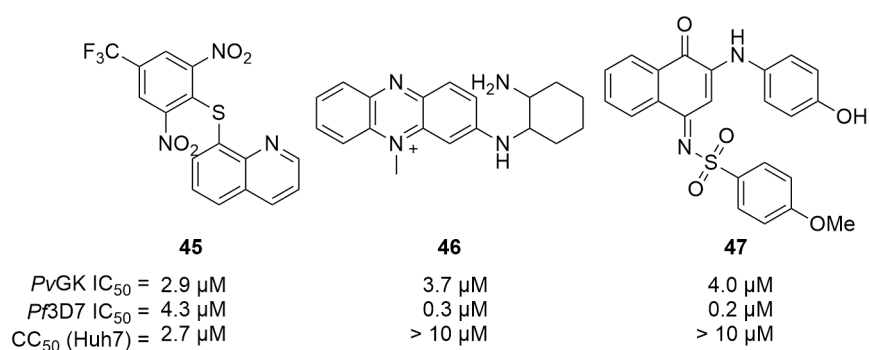


Figure 12. Chemical structure of emodin.

4.7. Molecules Targeting *Plasmodium falciparum* Guanylate Kinase (*PfGK*)

Guanylate kinase catalyzes the transformation of (deoxy)guanosine-monophosphate into (deoxy)guanosine-diphosphate. However, this transformation can potentially be bypassed by *P. falciparum* thymidylate kinase, which can also catalyze the *PfGK* reaction [66]. In their screening [46], Crowther et al. included guanylate kinase as one of their screened proteins but used *PvGK* (possibly because guanylate kinase expression is greater at liver stage [67]). Three compounds emerged as potential hits (**45** to **47**, Figure 13), but as the authors stated, structural features do not currently make them good candidates for further medicinal chemistry studies.

Figure 13. Inhibitors of *PvGK*.

4.8. Molecules Targeting *Plasmodium falciparum* Glycogen Synthase Kinase 3 (*PfGSK3*)

PfGSK3 is possibly an essential enzyme for the parasite asexual blood-stage [17] but its implication in biological pathways is not clear. One of its phosphorylation targets, the apical membrane antigen 1 (AMA1) [68], is required for RBC invasion by merozoites. *PfGSK-3* possesses a human ortholog, *HsGSK3*. *HsGSK3* hyperactivation is linked to Alzheimer's disease, diabetes, or cancers and many compounds inhibiting *HsGSK3* have been described [69].

In 2013, Fugel et al. realized a high-throughput screening of 10,000 molecules on *PfGSK3* [70]. Multiple molecules with a thieno[2,3-*b*]pyridine core were found to be hits against *PfGSK3*. Forty-three analogs from four different thieno[2,3-*b*]pyridines scaffolds were synthesized. The 3,6-diamino-4-(2-halophenyl)-2-benzoylthieno[2,3-*b*]pyridine-5-nitriles showed the best results on *PfGSK3* and *Sus scrofa* GSK3 (used for selectivity comparison). *HsGSK3* IC₅₀ and *PfNF54LUC* EC₅₀ tests were done on the best compound, **48** (Table 7).

Table 7. Thieno[2,3-*b*]pyridine inhibitors of *PfGSK3*.

Compound	R	% Inhibition <i>PfNF54LUC</i> at 3 μM	<i>PfGSK3</i> IC ₅₀ (μM)	<i>HsGSK3</i> IC ₅₀ (μM)	<i>PfNF54LUC</i> IC ₅₀ (μM)	CC ₅₀ (HEK293, μM)
48		n.d. ¹	0.24	9.08	5.5	35.2
49		99.9	0.72	40.2	1.2	5.56
(+)- 50		73.0	n.d.	n.d.	1.1	n.d.
(-)- 50		-24.4	n.d.	n.d.	n.d.	n.d.

¹ n.d. = not determined

Based on a docking study of **48** on an analogy model of *Pf*GSK3, Masch et al. synthesized 23 analogs of **48** with different substituents (halogens, ethers, alkylated amines, cyclic amines) at position 4 of the 2-chlorophenyl cycle [71] (Table 7). Compound **49** showed lower activity on *Pf*GSK3 but its potency on in vitro parasites was improved compared to **48**. Aqueous solubility was improved, going from 1.5 μM for **48** to 4.8 μM for **49**. The authors explored the possible axial chirality created by the 2-chlorophenyl cycle. They isolated the two isomers of **50** and compared their activity: (+)-**50** was active but not (–)-**50**. According to the authors, this difference arose from the chlorine atom position in (–)-**50**, which prevented it from fitting into the protein binding pocket.

4.9. Molecules Targeting *Plasmodium falciparum* Hexokinase (*Pf*HK)

Hexokinase is the first enzyme implicated in glycolysis, catalyzing the transformation of glucose into glucose 6-phosphate. Studies showed that *Pf*HK inhibition (directly or indirectly by inhibiting glucose transporters) is linked to parasite death during the asexual blood-stage [72,73]. *Pf*HK only possesses 24% similarity with human glucokinase, suggesting the possibility of designing selective *Pf*HK inhibitors [74].

In 2013, Harris et al. screened a small library of compounds on *Pf*HK [74]: three simple benzothiazolinones (**51–53**, Table 8) and the closely-related selenium compound ebselen (**54**) showed activity against *Pf*HK below 300 nM. These values were not completely correlated regarding EC_{50} against *Pf*3D7, as stated by the authors, who suggested that cell permeability and off-target interaction problems might explain the difference.

Table 8. Isobenzothiazolinones and ebselen as inhibitors of *Pf*HK.

Compound	X	R ₁	R ₂	<i>Pf</i> HK IC ₅₀ (nM)	<i>Pf</i> 3D7 EC ₅₀ (μM)
51	S	F	Cl	160	0.62
52	S	H	Cl	270	1.1
53	S	H	H	230	5.2
54	S	H	H	10	6.8

In another screening from the same team, Davis et al. screened 50,000 compounds on *Pf*HK [75]: two compounds (**55** and **56**, Table 9) displayed a micromolar activity on *Pf*HK but lacked activity on *Pf*3D7 in vitro.

Table 9. Dimethyl 2-amido-cyclopenta[*b*]thiophene-3,4-dicarboxylates as inhibitors of *Pf*HK.

Compound	R	<i>Pf</i> HK IC ₅₀ (μM)
55		1.09
56		1.94

4.10. Molecules Targeting *Plasmodium falciparum* Mitogen-Activated Protein Kinase 2 (PfMAP2)

Mitogen-activated protein kinases (MAPK) are proteins involved in signal transduction and are the key to many cellular processes in eukaryotic organisms. Two MAPKs are found in *P. falciparum*: PfMAP1 and PfMAP2. Very recently, Hitz et al. defined the role of these two proteins: neither is essential for asexual development, but PfMAP2 is essential for exflagellation, a mechanism that appears to be directly linked to this protein without the involvement of PfMAP1 [76]. Brumlik et al. screened some Hsp38 α (a human MAPK) inhibitors to assess their activity on *P. falciparum* [77]. One compound (57) displayed sub-micromolar activity on PfW2 (Figure 14).

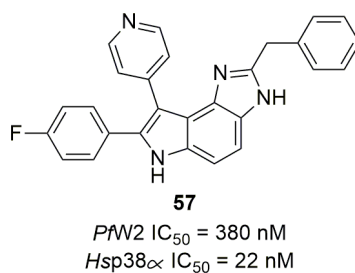


Figure 14. Inhibitor of Pfmap-2 discovered by Brumlik et al. [77].

4.11. Molecules Targeting *Plasmodium falciparum* MO15-Related Protein Kinase (PfMRK)

PfMRK is a cyclin-dependent kinase located in the nucleus. It interacts with two other proteins [78]: CDK-activating kinase assembly factor (PfMAT1) and cyclin 1 (Pfcyc-1). These three proteins are considered likely to be important for gene expression and DNA replication, and PfMRK is believed to be essential for the asexual blood-stage [17]. PfMRK shares the most properties, regarding human proteins, with HsCDK7.

Woodard et al. screened 12 isoquinoline and naphthalene sulfonamides, known for being kinase inhibitors, on PfMRK [79]. Only 58 displayed a sub-micromolar activity on PfMRK but was not active on *P. falciparum* in vitro. The same team later designed some chalcones based on an in-silico model of HsCDK7 [80]. One of these chalcones, 59, displayed a 1.3 μ M activity against PfMRK associated with an in vitro activity of 4.6 μ M on PfW2. Caridha et al. tested 27 sulfonamide compounds substituted by phenyl or thiophene cycles [81]. Ten molecules, including compound 60 (Figure 15), possessed a sub-micromolar activity on PfMRK, but none was active on PfW2. Cytotoxicity of compound 60 on five different cell lines was assessed, and all the values were around or above 25 μ M.

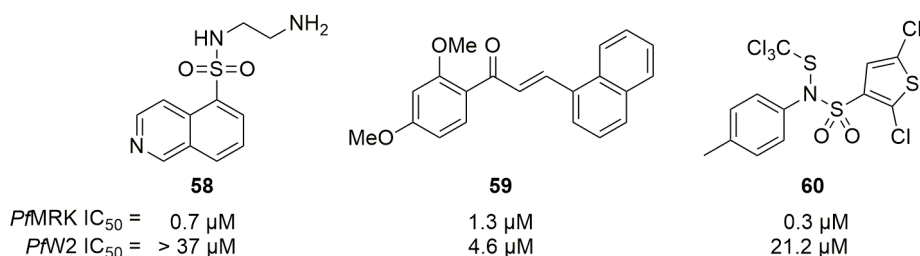


Figure 15. Inhibitors of PfMRK.

4.12. Molecules Targeting *Plasmodium falciparum* NIMA-Related Kinase 1 (PfNEK1)

Never-in-mitosis/Aspergillus (NIMA) related protein kinases are found in multiple eukaryotic organisms and are involved in the cell division process. Four NEK proteins have been discovered in *Plasmodium* [82]:

- PfNEK2 and PfNEK4 are related to sexual stages of the parasite,

- *PfNEK1* and *PfNEK3* can interact with *PfMAP2* and,
- *PfNEK1* is also expressed during schizogony (it is the only NEK essential in the blood-stage) and in male gametocytes.

Laurent et al. and Desoubzdanne et al. found two natural compounds targeting this protein (Figure 16): xestoquinone **61** and alisiaquinone **62** [83,84] displayed interesting in vitro parameters and an in vivo activity on a mouse model at 5 mg/kg during a four-day Peter's test [27]. Xestoquinone **61** was found to be selective of *PfNEK1* versus other plasmodial and human kinases.

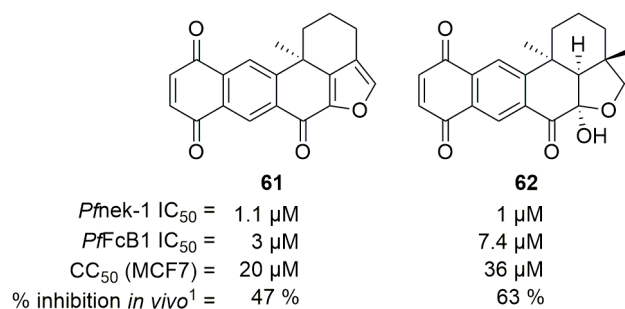


Figure 16. Natural quinone inhibitors of *Pfnek-1*. ¹ 4-day Peter's test at 5 mg/kg/4 days intraperitoneal.

4.13. Molecules Targeting *Plasmodium falciparum* Phosphatidyl-Inositol 4-Kinase (*PfPI4K*)

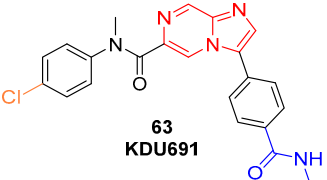
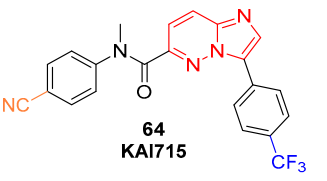
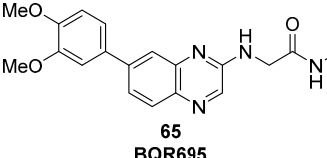
Phosphatidyl-inositol 4-kinase catalyzes the transformation of phosphatidyl-inositol into phosphatidyl-inositol 4 phosphate (PI4P). PI4P is a key secondary messenger involved in multiple cellular pathways. Inhibition of *PfPI4K* led to activity on every stage of the *Plasmodium* cycle [85] with inhibition of:

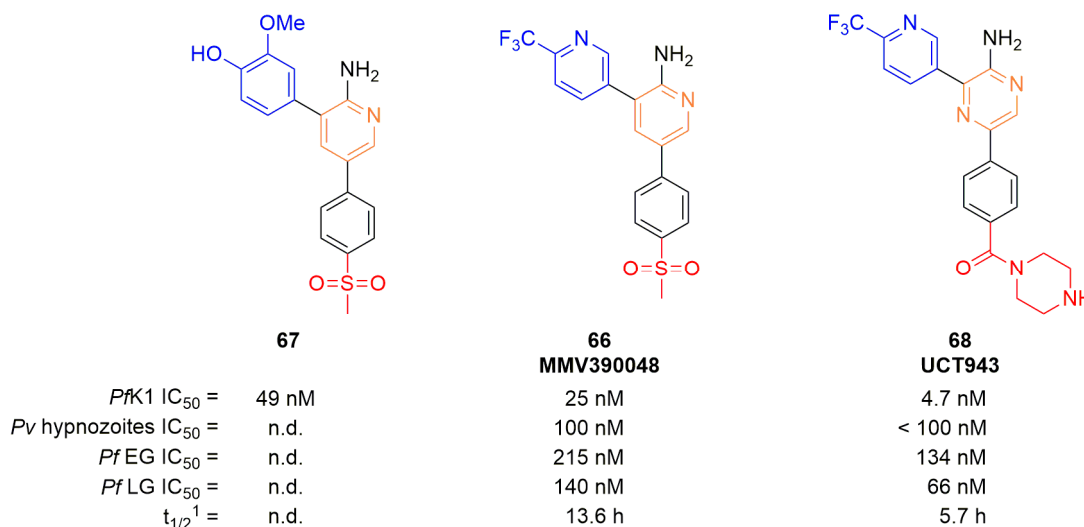
- Development of schizonts and hypnozoites in the liver stage,
- Asexual growth and gametocytogenesis in blood-stage,
- Reduction of oocysts in mosquito midgut in the sexual stage.

PfPI4K inhibition phenotypic consequences were found by McNamara et al. using four compounds as tools (three are showed in Table 10) [85]. The most assessed compound, KDU691 (**63**) an imidazopyrazine, showed promising in vivo activity on luciferase-expressing *P. berghei*-infected mice: with a single dose of 7.5 mg/kg, **63** was able to protect mice from what would be otherwise a fatal infection. **63** is selective of *PfPI4K* versus other human kinases including *HsPI4K* (IC₅₀ = 7.9 μM). Other tested compounds included the imidazopyridazine KAI715 (**64**) and the quinoxaline BQR695 (**65**). Their in vitro results are summed up in Table 10. *PfPI4K* was confirmed as the target of these compounds by analysis of resistant clones and artificial modifications of *PfPI4K* gene were also associated with drug resistance.

PfPI4K inhibitors are the most advanced plasmodial kinase inhibitors in terms of drug development. One compound, MMV390048 (**66**), is currently in phase II of clinical trials and is the only plasmodial kinase inhibitor currently in clinical trial (Figure 17) [86]. MMV390048 PI4K inhibitory effects, with the consequence of a multistage activity, were demonstrated by Paquet et al. using chemoproteomics studies [87]. This inhibition is selective, as *HsPI4Kα* and *HsPI4Kβ* were not targeted by **66**. Numerous in vivo experiments were performed (on mice, rats, dogs, and monkeys): multistage potency was conserved in in vivo models and **66** displayed very good pharmacokinetic parameters including bioavailability of 74% and a half-time of 66 h at an oral dose of 5 mg/kg on monkeys. All these parameters pointed **66** as a strong lead compound candidate for clinical studies for single exposure radical cure; it could also be promising for chemoprevention. Compound **66** originally emerged from a SAR study by Younis et al. which, starting from compound **67**, focused on modulation of the methoxyphenol group [88].

Table 10. Three of the inhibitors of *Pf*PI4K described by McNamara et al. [85].

Compound	<i>P. yoelii</i> liver stage IC ₅₀ (nM)	<i>P. falciparum</i> Asexual Blood-Stage IC ₅₀ (nM)	<i>Pf</i> NF54 Gametocyte III IC ₅₀ (nM)
 <p>63 KDU691</p>	36	29.9	220
 <p>64 KAI715</p>	9	3.9	n.d. ¹
 <p>65 BQR695</p>	n.d.	71	n.d.

¹ n.d. = not determined**Figure 17.** Starting from **67**, medicinal chemistry work led to **66** which was further improved to **68**. EG = Early-stage gametocytes, LG = Late-stage gametocytes. ¹ mouse model, 20 mg/kg per os.

Initial human clinical studies on **66** “showed high variability in exposure, which was attributed to low aqueous solubility” [89]. To solve, this problem, Brunshwig et al., synthesized UCT943 (**68**), an analog of **66** where the methylsulfonyl was replaced by a piperazinyl methylcarbamoyl and the aminopyridine by an aminopyrazine to improve aqueous solubility [89]. This second generation of *Pf*PI4K inhibitor showed improved parameters on every stage of the malaria cycle. ADMET properties were impacted by the modification but the in vivo activity was conserved.

Continuing on this new aminopyrazine scaffold, Gibhard et al., synthesized two aminopyrazines (Figure 18) to explore a potential prodrug strategy by conversion of a sulfoxide into a sulfone [90]. Compounds **69** (pyrazine analog of **66**) and **70** showed nanomolar activities in vitro. Solubility was better at physiological pH for the sulfoxide derivative compared to the sulfone. In vivo, the 90%

effective dose (ED₉₀) per os was at 0.12 mg.kg⁻¹ for both compounds compared to 0.57 mg.kg⁻¹ for **66** and 0.25 mg.kg⁻¹ for **68** (on *Pf3D7* infected mouse model). As expected by the authors, **70** was found to be rapidly converted in vivo into **69**, validating the sulfoxide prodrug approach for this series of compounds.

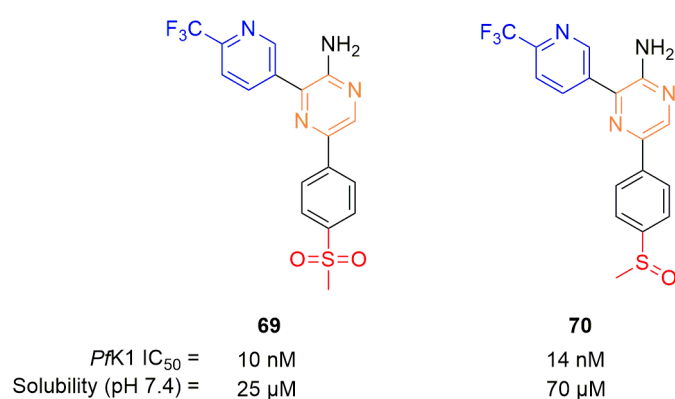


Figure 18. Aminopyrazines derivatives used to study a sulfoxide-to sulfone prodrug approach.

Other compounds with original scaffolds have been discovered since the development of **66**. Kandepedu et al. described a series of 1,5-naphthyridines, most of them having submicromolar activities on *PfNF54* [91]. Starting from MMV024101 (**71**), a SAR study was performed on the two substituents of the naphthyridine core (Figure 19) and 48 analogs were synthesized. Compound **73** showed good activity on *PfNF54* with an IC₅₀ of 63 nM and good metabolic stability ($t_{1/2}$ = 33 h at 5 mg/kg per os) but limited oral bioavailability (39%) on a mouse model.

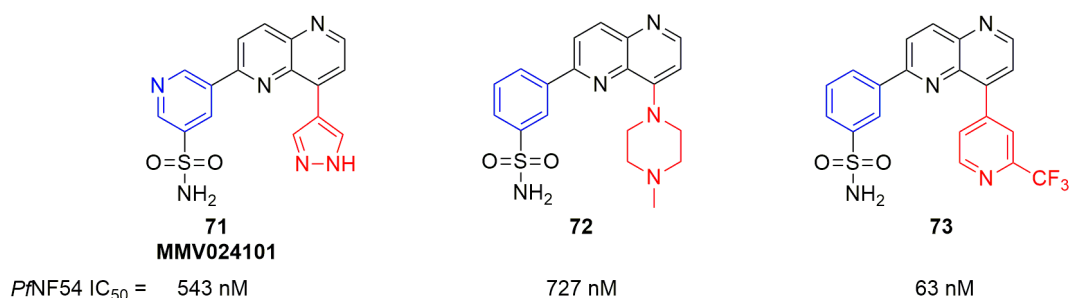


Figure 19. 1,5-naphthyridines as inhibitors of *PfPI4K*.

Liang et al. described a bipyridine series targeting *PfPI4K* from screening in their compound library [92]. Compound **74** displayed an activity around 4 μM on *Pf3D7* but was able to inhibit *PfPI4K* with an IC₅₀ of 7.7 nM (Figure 20). An in-silico homology model of *PfPI4K* was then used to rationally guide modulations on **74**: the 2-chloro-3-sulfonamide pyridine core was kept and modulations were performed on the two side chains. Compound **75** displayed the best activity on *Pf3D7* of 25.1 nM together with an IC₅₀ on *PfPI4K* of 0.9 nM. This activity was shown to be selective of *PfPI4K* versus human kinases including *HsPI3K* or *HsPI4K*. With oral bioavailability of 80% and a $t_{1/2}$ of 3.6 h on a mouse model (at 10 mg/kg per os), **75** was able in vivo to cure blood-stage *P. yoelii* at 80 mg/kg/7 days and to stop liver-stage infection by *P. berghei* sporozoites at a single dose of 1 mg/kg in a mouse model.

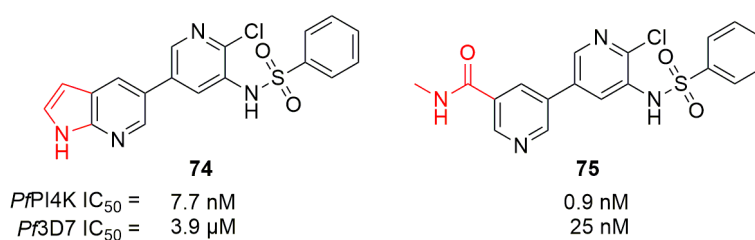


Figure 20. 2-chloro-3-sulfonamide pyridines as inhibitors of *PfPI4K*.

4.14. Molecules Targeting *Plasmodium falciparum* Protein Kinase 5, 6, 7 and 9 (*PfPK5*, *PfPK6*, *PfPK7* and *PfPK9*)

PfPK5 is a cyclin-dependent-like kinase [93], essential for the parasite blood-stage [17], with functions still unclear. Because of its homology with *HsCDK2* [94], *PfPK5* could be linked to cell division regulation [95]. *PfPK6* is another cyclin-dependent like kinase most likely essential for the asexual blood-stage [17,18,96]. *PfPK7* is an orphan kinase (it does not cluster with defined eukaryotic kinase groups) distantly related to the MAPKK proteins (mitogen-activated protein kinase) [97]. Disrupting *PfPK7* showed that it was not essential for the asexual blood-stage and was linked to a reduced growth rate during the asexual blood-stage and a lower number of oocysts in the mosquito midgut [98]. *PfPK9* is another orphan kinase, essential for the blood-stage [17], with currently only one known phosphorylation target: the E2 ubiquitin-conjugating enzyme 13 (*PfUBC13*) [99]. The human homolog, *HsUBC13*, is involved in DNA repair and immune responses [100].

4.14.1. Molecules Targeting *Plasmodium falciparum* Protein Kinase 5 (*PfPK5*)

Compound **76** is a known *HsCDK2* and *PfPK5* inhibitor, but is 1000 times more potent toward the human protein (Figure 21). Eubanks et al. synthesized analogs of **76** to try to obtain selective compounds against *PfPK5* but their modifications failed [95]. Since *PfPK5* crystal structure was available, authors also realized an in-silico screening of 35,000 compounds against *PfPK5* followed by an in vitro high throughput binding screening. A chromen-2-one-based compound was found interesting and analogs of this compound were purchased and tested. Compound **77** was the most interesting analog, with a $K_{d(\text{app})}$ of 3.8 μM and 5 μM against *PfPK5* and *HsCDK2* respectively but lacked activity against *PfDd2* in vitro (Figure 21).

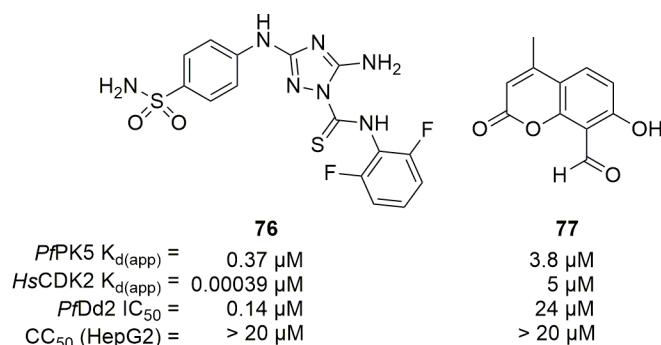


Figure 21. Inhibitors of *PfPK5* discovered by Eubanks et al. [95].

4.14.2. Molecules Targeting *Plasmodium falciparum* Protein Kinase 6 (*PfPK6*)

PfPK6 inhibitors have only been identified as hit compounds in the screening by Crowther et al. Figure 22 displays two compounds (**78** and **79**) having an IC₅₀ against *PfPK6* of around 60 nM but lacked selectivity toward *PfPK6*.

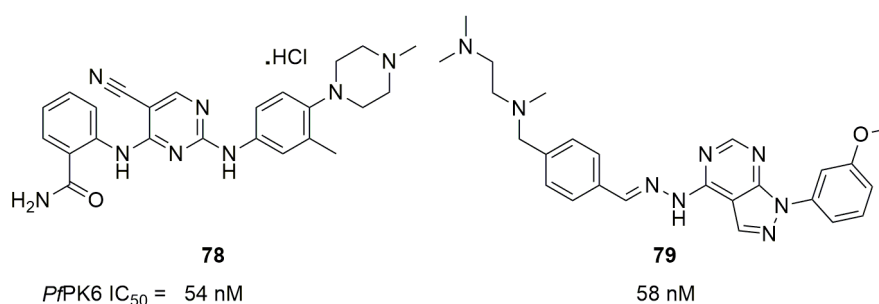


Figure 22. Molecules inhibiting *PfPK6* discovered by Crowther et al. [33].

4.14.3. Molecules Targeting *Plasmodium falciparum* Protein Kinase 7 (*PfPK7*)

Starting from hit compound **80** identified by a high-throughput screening campaign, Bouloc et al. synthesized a series of 3-amino-6-phenyl-imidazopyridazines (Figure 23) [101]. Thirty-five analogs were synthesized with modulations targeting the substitution of the amine at position 3 and the nature of the *para*-substituent on the phenyl at position 6. Compound **81** was the best compound of this series, with an IC₅₀ of 0.13 μ M and 1.09 μ M against *PfPK7* and *Pf3D7* respectively.

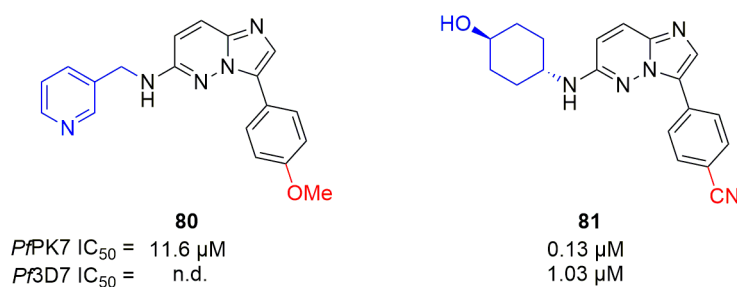


Figure 23. Imidazopyridazine inhibitors of *PfPK7*.

Merckx et al. realized a screening of compounds against *PfPK7*, where two molecules (**82** and **83**, Figure 24) with an imidazopyridazine or pyrazolopyrimidine core displayed moderate activity on both kinase and *Plasmodium* inhibition assays [102]. Klein et al. tested their series of pyrazolopyrimidines against *PfPK7* [103]. Compound **84** was shown to inhibit *PfPK7* at 10 μ M by approximately 60% (Figure 24).

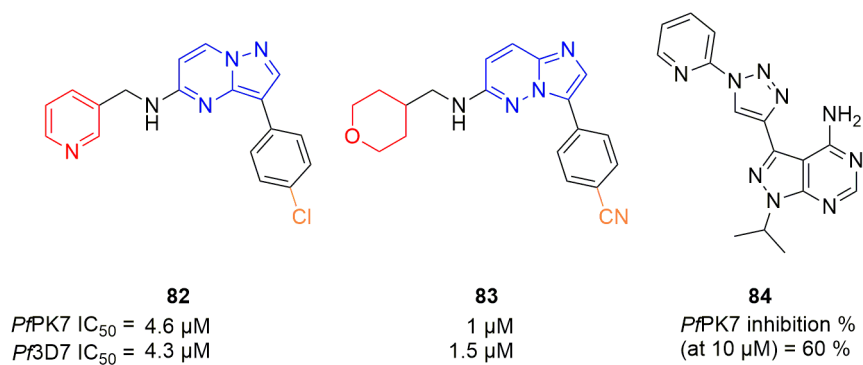
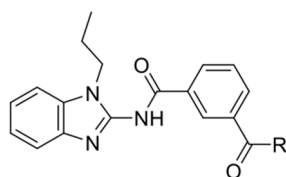


Figure 24. Hits inhibiting *PfPK7* discovered by Merckx et al. and Klein et al. [102,103].

4.14.4. Molecules Targeting *Plasmodium falciparum* Protein Kinase 9 (PfPK9)

PfPK9 inhibitors have only been explored by Raphemot et al. [100]. Starting from a screening of 3,200 molecules, authors discovered that taketinib (85), an inhibitor of HsTAK1, was an inhibitor of PfPK9 (Table 11). 85 used at its EC₅₀ on *P. berghei* infected HuH7 cells (7.3 μM) was able to increase the size of intracellular parasites but not their numbers, suggesting a deregulation of growth pathways by inhibition of PfPK9. Analogs of taketinib were synthesized to obtain selective compounds against PfPK9: analog 86 displayed a decreased affinity toward PfPK9 at 4.1 μM but was unable to interact with HsTAK1 (Table 11).

Table 11. Taketinib and its analog as inhibitors of PfPK9.



Compound	R	K _{d(app)} PfPK9 (μM)	HuH7 <i>P. berghei</i> Infected Cell Load EC ₅₀ (μM)
85 (taketinib)	NH ₂	0.46	7.3
86	OH	4.1	43

4.15. Molecules Targeting cGMP Cyclin-Dependent Protein Kinase (PfPKG)

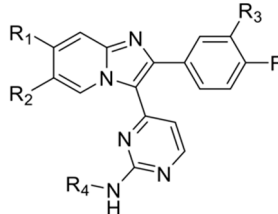
PfPKG is a serine/threonine kinase involved in mechanisms at all the stages of parasite life: parasite motility, hepatocyte invasion, asexual blood-stage development, and gametocytogenesis [104–108]. Compared to HsPKG, PfPKG possesses a smaller gatekeeper (the amino acid at the entrance of the catalytic domain), making it easier to develop molecules selective toward PfPKG. Inhibition of PfPKG led to multistage activity [109,110] with inhibition of:




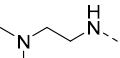
- sporozoite invasion during the liver-stage,
- development of asexual blood-stage,
- development of gametocytes,
- exflagellation in the mosquito midgut.

Two different scaffolds were explored for PfPKG inhibitors: imidazopyridines and trisubstituted five-membered aromatic cycles which could be viewed as ring-simplified analogs of the first series.

4.15.1. Imidazopyridines Targeting PfPKG

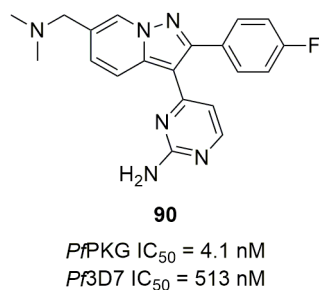
Starting from compound 87 (Table 12), designed originally as a PKG inhibitor against *Eimeria tenella* in chickens [111], Baker et al. realized a SAR study and synthesized nine analogs [110]. This work led to compound 88 with an IC₅₀ against PfPKG reduced from 3.1 to 0.16 nM and IC₅₀ against Pf3D7 strain reduced from 395 to 2.1 nM. 88 was also able to reduce blood-stage growth of *P. berghei* in a mouse model during a four-day Peter's test by around 60% (25 mg/kg/twice per day *per os*) [27]. Additional work to better understand the properties of the imidazopyridine series was carried out by Large et al., leading to compound 89 [112] which showed lower potency on *P. falciparum* than 87 but better selectivity and metabolic stability.

Table 12. Imidazopyridines as inhibitors of *Pf*PKG.


Compound	R ₁	R ₂	R ₃	R ₄	<i>Pf</i> PKG IC ₅₀ (nM)	<i>Pf</i> 3D7 IC ₅₀ (nM)	% MLM Remaining ¹
87		H	H	H	3.1	395	52
88		Me	-NHSO ₂ Me		0.16	2.1	-
89		H	H	Me	2.8	525	93

¹ % remaining after 30 min incubation with mouse liver microsomes.

Another study by Large et al. involved modifying the imidazopyridine core using a scaffold hopping strategy [113]. Compound **90**, a pyrazolopyridine analog of **87**, displayed activities on both *Pf*PKG and *Pf*3D7 similar to **87** but with lower metabolic stability (22% of compound remaining after 30 min incubation with mouse liver microsome) due to a more lipophilic molecule (Figure 25).

**Figure 25.** Pyrazolopyridine **90** as inhibitor of *Pf*PKG.

4.15.2. Trisubstituted Five-Membered Aromatic Cycles Targeting *Pf*PKG

Work on trisubstituted five-membered aromatic cycles started with Diaz et al., who tested compound **91** on *P. falciparum* (Figure 26) [114]. **91** was designed initially as an anticoccidial compound targeting *Eimeria tenella* PKG [115]. **91** displayed nanomolar activity against multiple recombinant *Pf*PKG and an IC₅₀ of 0.49 μM against *P. falciparum* NF54 strain. Activity in vivo on a mouse model infected by *P. berghei* was also assessed. At 50 mg/kg/7 days by intraperitoneal injections, **91** was able to delay the death of all the mouse groups by 12 days.

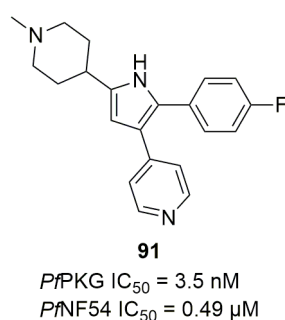


Figure 26. Trisubstituted pyrrole as inhibitor of *Pf*PKG.

Tsagris et al. decided to change the pyrrole core in **91** into a thiazole while keeping key substituents from both compounds **87** and **91** [116]. The authors started from compound **92** which displayed nanomolar inhibition of *Pf*PKG but lacked good potency in vitro against *Pf*3D7 (Figure 27). 15 analogs were synthesized, with modifications targeting all three substituents, leading to compound **93**. Using the same substituents as **87** led to improved potency but **93** displayed an *h*ERG IC₅₀ of 1.3 μM (Figure 27). **93** was assessed against human kinases and inhibited all the tested kinases below 70% at 100 nM. Starting from **93**, Matralis et al. continued pharmacomodulations, mainly on the *N*-substituent of aminopyrimidine and the replacement of *N*-methypiperidine [117]. Multiple compounds showed improved potency, including compound **94** (Figure 27). **94** was able to kill parasites rapidly, like artemisinin. Thus, the authors decided to assess **94** in a binding assay against other plasmodial kinases and found that it was also able to bind *Pf*CLK2. This dual activity was linked to the fast-killing profile, since compounds only targeting *Pf*PKG possessed a slow killing profile. Moreover, **94** also showed an affinity toward *Pf*CDPK1 and *Pf*CDPK4.

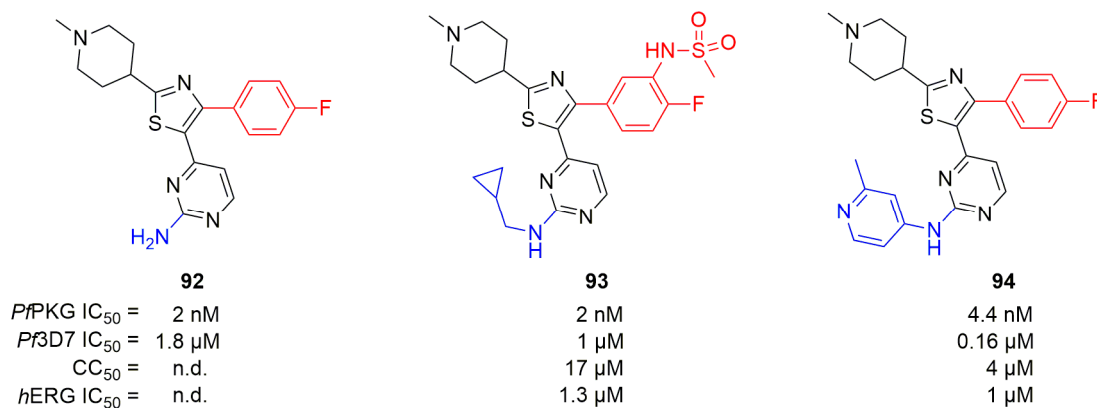


Figure 27. Trisubstituted thiazoles as inhibitors of *Pf*PKG.

From three isoxazole-based hits on *Pf*PKG of their own compounds' library, Ul Mahmood et al. explored the three positions on the isoxazole cycle while keeping key features like aminopyrimidine from previously described *Pf*PKG inhibitors [118]. 29 analogs were synthesized and tested on *Pf*PKG: six of them displayed an IC₅₀ below 50 nM, including compound **95**, the most potent one (Figure 28). Finally, MMV030084 (compound **96**, Figure 28), a trisubstituted imidazole, was described by Vanaershot et al. as a multistage active compound (no gametocytocidal effect) targeting *Pf*PKG [109]. Using knockout studies, *Pf*PKG was confirmed to be the main target of **96**.

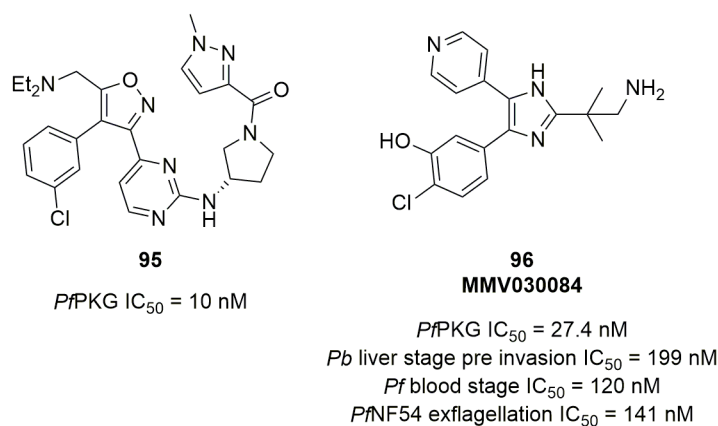


Figure 28. Trisubstituted isoxazole and imidazole as inhibitors of PfPKG.

4.16. Molecules Targeting Plasmodium falciparum Thymidylate Kinase (PfTMK)

PfTMK is the enzyme catalyzing the phosphorylation of thymidine monophosphate to thymidine diphosphate. PfTMK is involved in the de novo synthesis of purine bases and is the only way for the parasite to create these bases [119]. This pathway also includes major drug targets such as dihydrofolate reductase (PfDHFR, targeted by sulfadoxine) and more recently, dihydroorotate dehydrogenase (PfDHODH, targeted by DSM265 [120]). PfTMK is also able to catalyze the phosphorylation of deoxyguanosine monophosphate into deoxyguanosine diphosphate.

Research on PfTMK inhibitors has been centered on thymidine analogs. Cui et al. synthesized a large number of thymidine analogs bearing a urea side chain [121]. Compound 97 was used as a starting point for pharmacomodulations which led to compound 98 (Figure 29). Modulations showed several structural features important for potency: α -anomers were more potent than β , thiourea decreased potency whereas hydrophobic *para*-substituent on the phenyl urea increased potency. 98 displayed nanomolar activity on Pf3D7 but with low microsomal stability. Surprisingly, no inhibitory assays were performed on the most potent compounds of the series, meaning no clear conclusion can be drawn on the target of these compounds.

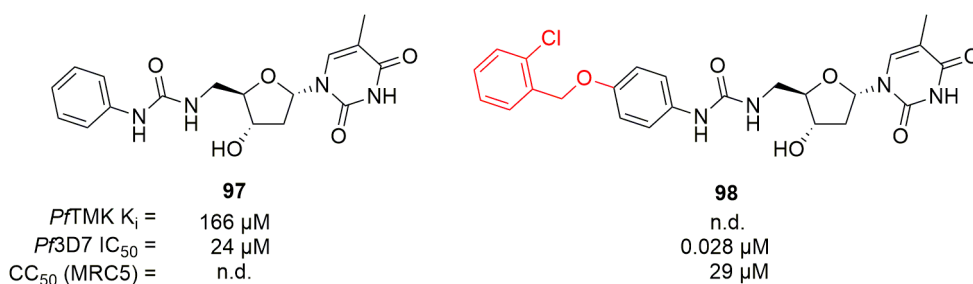


Figure 29. α -thymidine urea analogs as inhibitors of PfTMK.

Simpler structures were explored by Kato et al., who replaced the tetrahydrofuran by a cyclopentene [122]. This led to compound 99, showing a weak activity with a K_i of 20 μ M on PfTMK (Figure 30). 99 was then used by Noguchi et al. as a starting point for pharmacomodulations on the side chain of the cyclopentene core [123]. Compound 100 with a fluoroethanol substituent displayed better potency than 99, with a K_i of 14 μ M (Figure 30).

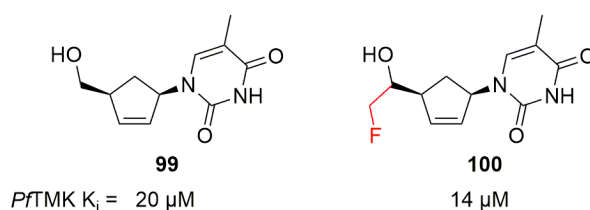


Figure 30. Cyclopentene analogs of thymidine as inhibitors of *PfTMK*.

5. Conclusions

Plasmodial kinases appear to be promising targets for new drug therapies against malaria. In the best cases, their inhibition can impact every stage of the parasitic cycle. This is due to the involvement of kinases in most of the cellular mechanisms. Zhang et al. created a mutagenesis index score (MIS) for each gene of the *P. falciparum* asexual blood-stage, illustrating the essentialness of each of these genes (Figure 31) [124]: only five of the kinases described in this review are considered dispensable (MIS close to 1) but two of them (*PfCDPK4* and *PfPK7*) are known to be key proteins during exflagellation.

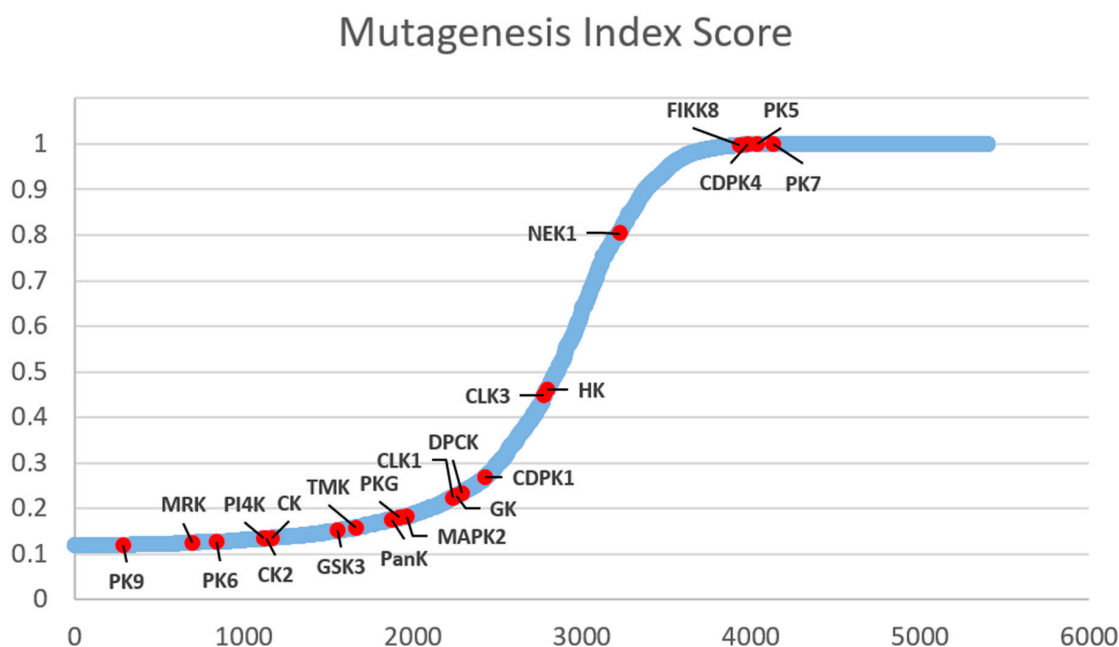


Figure 31. Mutagenesis Index Score graph with protein kinases described in the review highlighted. All *P. falciparum* genes were ordered from the lowest to the highest score and then attributed an arbitrary rank number to obtain this curve. Data from Zhang et al. [124].

MMV390048 (**66**), targeting *PfPI4K* is currently the only plasmodial kinase inhibitor in clinical trial. However, this could quickly change as the way is opened to other *PfPI4K* inhibitors. This makes *PfPI4K* inhibitors the most advanced plasmodial kinase inhibitors in the drug development process (Table 13). *PfCDPK4* inhibitors are getting close to a pre-clinical stage while *PfCDPK1*, *PfCLK3*, *PfGSK3* and *PfPKG* inhibitors are promising compounds awaiting further studies. This represents only six out of the 22 kinases described in this review. Many are still in a hit discovery stage, sometimes without progress for more than ten years.

Table 13. Plasmodial kinases targeted by inhibiting compounds described in this review.

Protein	Role of Protein—Consequence(s) of Inhibition ¹	Development Stage of the Most Advanced Compound(s)	Studies Reviewed
CDPK1	Egress and invasion processes regulator—Inhibition of asexual blood-stage growth and gametogenesis, reduced mosquito infection	Hit to lead	[23,24,28–33]
CDPK4	Exflagellation regulator—Reduced mosquito infection	Lead optimization	[33,36–39]
CK	Transformation of choline into phosphocholine	Hit discovery—Target validation needed	[41,45–47]
CK2	Serine/threonine kinase, possibly phosphorylating many proteins during asexual blood-stage	Hit discovery—In vitro activity to be determined	[50]
CLK1	Phosphorylation of spliceosomes proteins—Inhibition of asexual and sexual blood-stage growth	Hit discovery—In vitro activity to be determined	[53,55]
CLK3	Phosphorylation of spliceosomes proteins—All-stage activity	Hit to lead	[53,54,56]
PanK	Transformation of pantothenate into 4'-phosphopantothenate	Hit discovery	[58–60]
DPCCK	Transformation of dephospho-coenzyme A into coenzyme A	Hit discovery	[61]
FKk8	Not defined	Hit discovery	[64,65]
GK	Transformation of (deoxy) guanosine-monophosphate into (deoxy) guanosine-diphosphate	Hit discovery—Target validation needed	[46]
GSK3	Not defined	Hit to lead	[70,71]
HK	Transformation of glucose into glucose-6-phosphate	Hit discovery	[74,75]
MAP2	Involved in many key cellular processes—Inhibition of asexual blood-stage and exflagellation	Hit discovery—Target validation needed	[77]
MRK	Regulation of gene expression and DNA replication	Hit discovery	[79–81]
NEK1	Regulation of cell division processes	Hit discovery	[83,84]
PI4K	Transformation of phosphatidyl-inositol into phosphatidyl-inositol 4 phosphate—All-stage activity	Phase II clinical trial	[85,87,89–92]
PK5	Cyclin-dependent like kinase, possibly regulating cell division	Hit discovery	[95]
PK6	Cyclin-dependent like kinase, role not defined	Hit discovery - In vitro activity to be determined	[33]
PK7	Orphan kinase, role not defined—Inhibition of asexual blood-stage and reduced mosquito infection	Hit to lead	[101–103]
PK9	Orphan kinase, role unclear, able to phosphorylate <i>Pf</i> UBC13—Deregulation of asexual hepatic stage growth	Hit discovery	[100]
PKG	Serine/threonine kinase—All-stage activity	Hit to lead	[109,110,112–114,116–118]
TMK	Transformation of thymidine monophosphate into thymidine diphosphate	Hit to lead	[121–123]

¹ When not specified, each of these proteins is assumed to at least inhibit asexual blood-stage growth.

The development of plasmodial kinase inhibitors is slowed by a lack of X-ray structural data, challenges regarding selectivity against human kinases, and loss of potency going from protein to parasite activity. Moreover, new antiplasmodial hit molecules without a defined target usually have their mechanism of action explored based on commercial antiplasmodial drugs. If these compounds possess multistage activity, they should be tested on plasmodial kinases.

There is still plenty of room for new kinase inhibitors: only 22 out of 86 (or 99) estimated plasmodial kinases were found to be targeted by the compounds in this review. In addition to plasmodial kinase inhibitors, other new promising drugs in development targeting plasmodial proteins such as *PfDHODH* or *PfATP4* will be important for the creation of new powerful drug combinations to reduce numbers of malaria cases and deaths in years to come.

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