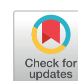
Drug targeting of one or more aminoacyl-tRNA synthetase in the malaria parasite *Plasmodium falciparum*



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Malaria remains a major infectious disease and, despite incidence reduction, it threatens resurgence in drug-resistant forms. Antimalarial drugs remain the mainstay of therapeutic options and hence there is a constant need to identify and validate new druggable targets. *Plasmodium falciparum* aminoacyl-tRNA synthetases (*Pf*-aaRSs) drive protein translation and are potent targets for development of next-generation antimalarials. Here, we detail advances made in structural-biology-based investigations in *Pf*-aaRSs and discuss their distribution of druggable pockets. This review establishes a platform for systematic experimental dissection of malarial parasite aaRSs as a new focus for sustained drug development efforts against malaria.

Introduction

Plasmodium falciparum causes malaria and accounts for the death of >0.4 million people annually (WHO malaria report, 2017 http://www.who.int/malaria/publications/world-malaria-report-2017/en/). Malaria has a tremendous adverse impact on the quality of life, overall health and economic status of those infected. Despite a reduction in overall incidence, malaria threatens resurgence because of resistance development against the top antimalarial drugs including artemisinin (WHO malaria report, 2016). Thus, there is a pressing need for identification and validation of new druggable targets against human malarial parasites like *P. falciparum* and *Plasmodium vivax*. It is noteworthy that any (most) new antimalarials would probably be administered as part of a cocktail therapy, possibly with one or two other partner drugs [1,2]. We, as well as other groups, have explored several malarial parasite protein families from structural and functional perspectives, including histone chaperones, kinases, fatty acid binders and adhesins [3–8]. However, the lack of experimentally verified 'hit' compounds has hampered the discovery of potent malarial parasite inhibitors against these *P. falciparum* protein targets. Since 2009, encouragingly, several groups have been investigating the structure–function attributes of the malarial parasite aminoacyl-tRNA synthetase (aaRSs) family in *P. falciparum* [9–22]. Targeting parasite aaRSs can provide an additional drug component in the current multidrug antimalarial therapy [9–23]. A recent example of success in targeting aaRSs comes from tavaborole (Kerydin®) – an FDA-approved antifungal drug that works on the editing domain of leucyl-tRNA synthetase against onychomycosis [24,25]. In this direction, malarial parasite protein translation components such as aaRSs provide a huge new resource of potential drug targets.

The aaRSs control protein biosynthesis pathways by allowing pairing of cognate tRNA with amino acids and a cellular translational compartment usually contains 20 aaRSs (Fig. 1). These enzymes are divided into two classes based on conserved motifs and topology in catalytic domains. Class I enzymes contain the ATP-binding motifs HIGH and KMSKS, whereas three conserved sequence motifs called 1, 2 and 3 are the characteristics of class II enzymes. In recent years, few *P. falciparum* aaRSs have been validated as new foci for drug development [14,23,26–33]. It is therefore worthwhile to pursue additional members of the *P. falciparum* aaRS family given that within these enzymes the

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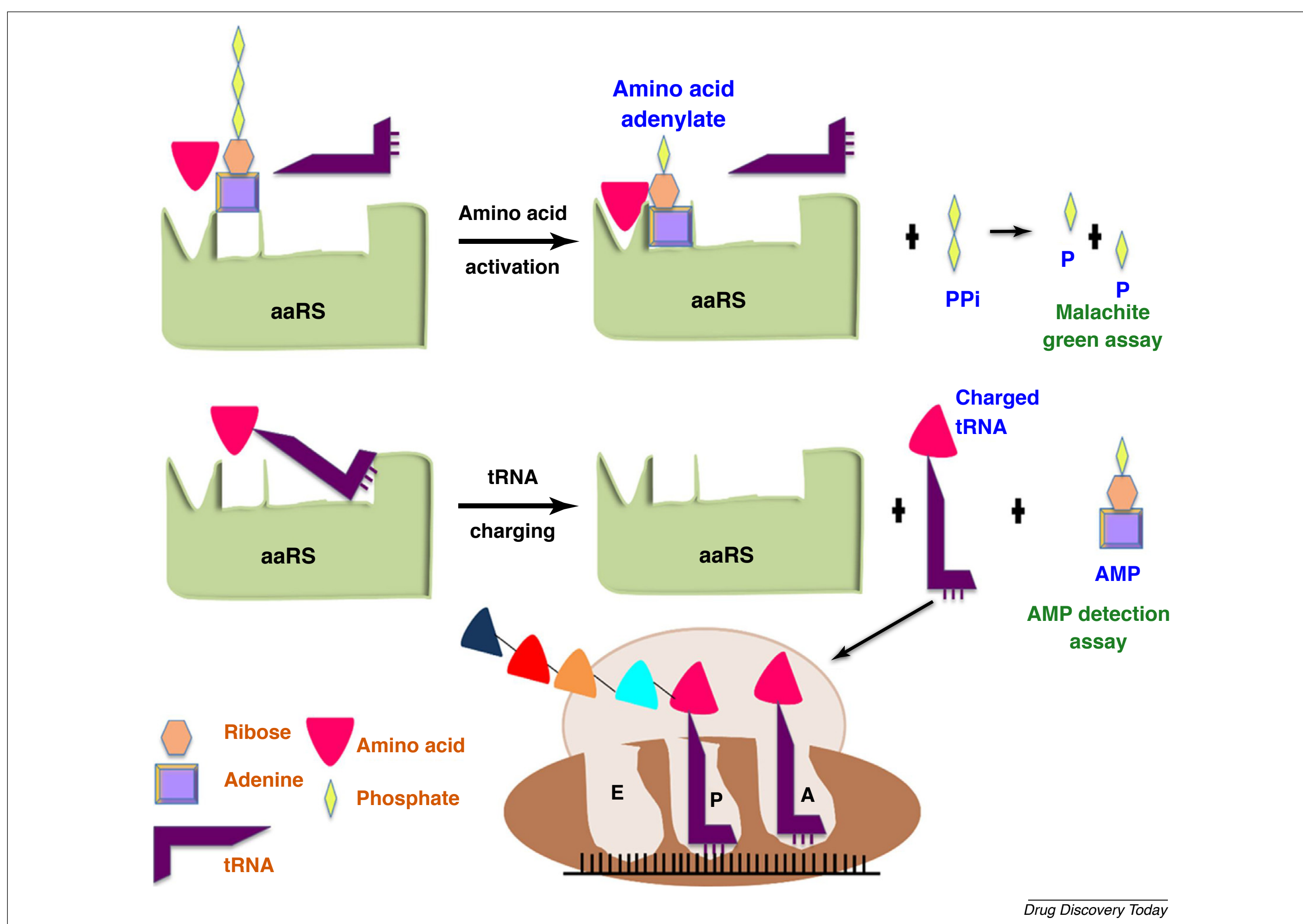


FIGURE 1
Aminoacylation reaction and enzymatic assay. In the first step, the aminoadenylate complex is formed with the release of pyrophosphate (PPi), which can be indirectly measured by a malachite green assay that uses pyrophosphatase-induced conversion of PPi into inorganic phosphate (Pi). In the second step, the aminoadenylate complex interacts with cognate tRNA, charges it and liberates AMP. This AMP can be measured by the AMP-glo^{TM} assay. The charged tRNA (tRNA-amino acid complex) is then utilized for protein translation.

overall structures and modes of action tend to be conserved yet druggable (Figs 1, 2). With several examples, here we highlight the opportunities for targeting all 20 members of the *P. falciparum* aaRS family that together collaborate to fulfill the essential protein translational requirements in the cytoplasm of *P. falciparum*. We have analyzed aaRS sequences from the five human-infecting malaria species of *P. falciparum, P. vivax, Plasmodium knowlesi, Plasmodium ovale* and *Plasmodium malariae*. We note that most of the cytoplasmic aaRSs display very high sequence identities across the mentioned five plasmodial species – for example the *Pf*-PRS^{cyto} shows a minimum of 90% identity across human malaria parasites. Our central thesis is that, because paralysis of any one of the 20 cytoplasmic aaRSs of *P. falciparum* abrogates protein synthesis and kills the parasite, additional *Pf*-aaRSs must also be equally worthy of drugging.

The *P. falciparum* aminoacyl-tRNA synthetase family

In 2009, we highlighted the whole spectrum of aaRSs in *P. falciparum* and proposed their experimental dissection from a drug discovery perspective [9]. Since then, numerous international groups including us have made significant advances in deepening the cumulative wealth of data on *Pf*-aaRSs [9,11,17,20,23–29,32,34]. Indeed, aaRSs can serve as valuable targets in other eukaryotic pathogens like *Toxoplasma gondii* and *Cryptosporidium parvum* [35,36]. All current data together suggest that the three *P. falciparum* chambers (i.e., cytoplasm, apicoplast and mitochondria) fulfill their protein translation requirements of charged tRNAs via the combined activities of the 36 *Pf*-aaRSs [37]. Whereas the apicoplast encodes and imports most protein translation motors including its complete set of aaRSs for generating charged tRNAs, the *P. falciparum* mitochondria seem reliant on importing the same from the parasite cytoplasm [37]. Using bioinformatics tools, cell biology and logical deduction, the complete spatial distribution of all 36 *Pf*-aaRSs has become clear – at least for the erythrocytic stages of the *P. falciparum* lifecycle (Fig. 2). Of a total of 36 *Pf*-aaRSs, 16 reside in the parasite cytoplasm, 15 in its apicoplast, four single-gene aaRSs (ARS, TRS, GRS and CRS) are shared between the cytoplasm and apicoplast [16,38,39] and one (mFRS) is mitochondrial within *P. falciparum* [37] (Fig. 2). This review will focus on the advances made in the understanding and

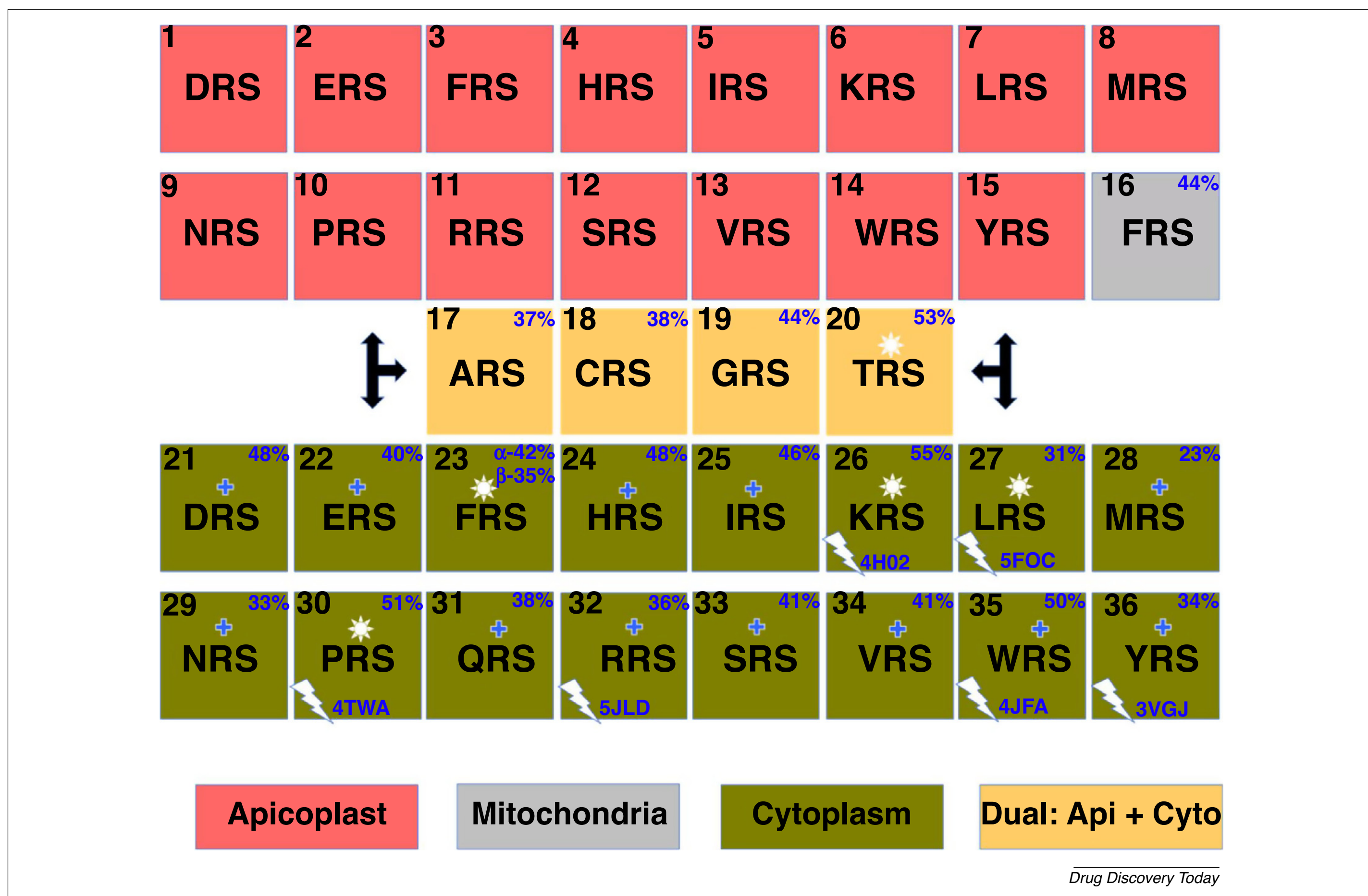


FIGURE 2

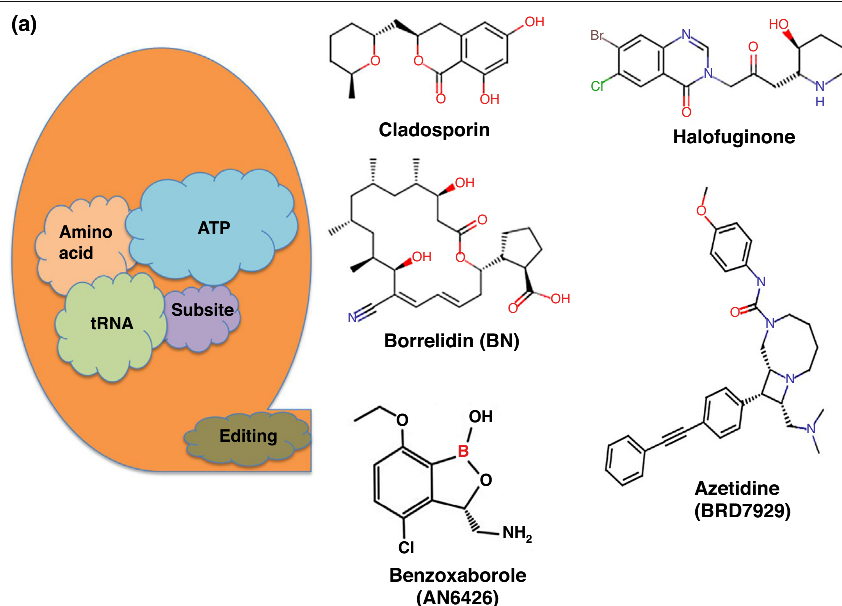
Pf-aaRSs periodic table. Each box in the periodic table represents one *Pf* aaRS (36 in total). The aaRSs are color-coded based on their cellular location: pink (apicoplast), green (cytoplasm), yellow (apicolpast and cytoplasm) and gray (mitochondria). The lightning mark represents those aaRS^{cyto} for which crystal structures are known, the star sign indicates where very potent inhibitors are known whereas the plus sign indicates *Pf*-aaRSs that deserve additional experimental interrogation. The PDB IDs of the known crystal structures are given in blue.

drug targeting of cytoplasmic versions of *Pf*-aaRSs because these seem to be most pertinent to antimalarial drug development.

Inhibition of *Pf*-aaRSs has so far been achieved by targeting the numerous subsites on these enzymes (Figs 2 and 3a). The key interactions revealed from crystal structures of *Pf*-aaRS–drug complexes, and from additional cases with highly conserved binding modes in homologous enzymes, reveal a very interesting scenario for drug targeting of aaRSs (Figs 2 and 3a). Specifically, for malaria parasite aaRSs, we have a deep understanding of enzymes that engage with small-molecule inhibitors like cladosporin (CL), halofuginone (HF), benzoxaboroles and borrelidin (BN) (Figs 2 and 3a). These potent inhibitors target the following subsites in aaRSs and in each case decimate parasite protein synthesis activity: ATP-binding site – CL mimics adenosine and binds in the ATP pocket of lysyl-tRNA synthetase [15,23]; amino acid and tRNA binding subsites – HF functions by binding prolyl-tRNA synthetase in an ATP-dependent manner and mimics L-pro and tRNA 3′-A76 [20,27]; the 3-aminomethyl benzoxaborole compound (AN6426) probably targets the *Pf*-LRS-editing domain by covalently inactivating the 3′ A76 nucleotide of tRNA and catalytic turnover [32]; the highly potent BN compound interacts with three prototypical substrate-binding subsites (of four that bind to BN) in threonyl-tRNA synthetase of orthologous TRSs [40] (Fig. 3b). The mode-of-action for azetidine which targets *Pf*-FRS^{cyto} remains to be structurally elucidated [31]. These diverse binding modes of *Pf*-aaRS inhibitors clearly indicate the plethora of combinations possible to inactivate these enzymes by designing specific inhibitors for different subsites (Fig. 3). Given the presence of numerous substrate-binding cavities per *Pf*-aaRS (usually >3), the possible combinations of subsites that can be blocked per aaRS is large – and hence the tremendous potential to exploit each aaRS individually or collectively.

Screening of drug-like libraries against *P. falciparum* aminoacyl-tRNA synthetases

Aminoacylation is a two-step reaction where aminoacyl adenylate is first formed using ATP and an amino acid where pyrophosphate (PPi) is released in the first step owing to ATP hydrolysis (Fig. 1). This is followed by the transfer of amino acid to specific tRNA, leading to the release of AMP and aminoacyl-tRNA (i.e., charged tRNA; Fig. 1). In conjunction with phenotypic screening, or as stand-alone projects, the identification and validation of potent drug-like small-molecules that can inhibit *Pf*-aaRSs will be a valuable effort. Several biochemical assays have been developed that



(b)

Scaffold and aaRS	tRNA pocket	Amino acid pocket	ATP pocket	Subsite	Editing site pocket	IC50 (nM)	EC50 (nM)	References
Cladosporin KRS			+			61 in 3D7	48 in 3D7	23
Halofuginone PRS	+	+				9 in 3D7	1 in 3D7	27
Benzoxaborole (AN6426) LRS					+	310 in W2	190 in 3D7	32
Borrelidin TRS	+	+	+	+		0.9-7	1.8 in FCR3	30
Azetidine (BRD7929) FRS						23	9 in Dd2	31

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FIGURE 3

Chemical structures, target sites and potency of known inhibitors. (a) Potent drug-like molecules and their target pockets within a prototypical aaRS. Cladosporin: ATP-binding site, halofuginone: amino acid pocket and 3′-end of tRNA pocket, borrelidin: ATP-binding site, amino acid pocket, 3′-end of tRNA pocket and a subsite, benzoxaborole: editing site. (b) Associated IC_{50}s and EC_{50}s are given where available along with *Plasmodium falciparum* strain information.

assess both steps of the aminoacylation reaction (Fig. 1), thus allowing low- or high-throughput tracking of potential aaRS inhibitors. Two popular aminoacylation activity protocols are: (i) determining the AMP produced from the second step of the enzymatic reaction (e.g., the AMP-Glo^{TM}kit); and (ii) a colorimetric, malachite-green-based assay that can assess the released PPi from the first step of the reaction – although this assay in the presence of tRNA indicates charging of tRNA as well [41]. These assays can detect even picomolar quantities of either AMP or PPi released from the reaction, respectively [41,42] (Fig. 1). The AMP-Glo^{TM}kit entails a two-step process and in the first step this assay halts the aminoacylation reaction, simultaneously removes the unutilized ATP and converts the produced AMP into ADP. In the second step, the reaction transforms converted ADP into ATP, generating a light reaction from this converted ATP using luciferase (luciferin) (Fig. 1). Malachite green is an economical dye that exploits the production of PPi from the first step of the aminoacylation reaction and correlates with enzyme activity (Fig. 1). The

malachite green solution (initially brown in color) forms a dark green color as soon as it comes into contact with inorganic phosphate (conversion of PPi to two phosphates is done by adding a pyrophosphatase enzyme) and gives intense absorption in the range 620–650 nm [41,42] (Fig. 1).

Using the above two assay protocols, we propose high-throughput testing of all potent small-molecule hits discovered by phenotypic screening against malaria parasites so far for assessment of their possible interactions with *Pf*-aaRSs. Such efforts will provide a wider set of starting drug-like scaffolds that can then be investigated further for identification of their mechanisms-of-action in terms of enzyme inhibition (IC_{50}) potency, binding modes, cell-based potency (EC_{50}), selectivity over human aaRS (comparison of IC_{50} and EC_{50}) and binding affinity (K_d values). These sets of biochemical data will also help establish a platform for studying more-vital issues regarding drug development such as generation of resistance mutations, possible increase in the copy number of the targeted aaRS or epigenetic changes as a function of drug pressure [23,43].

Case of cytoplasmic phenylalanyl-tRNA synthetase

Within the *P. falciparum* aaRS family, phenylalanyl-tRNA synthetases (FRSs) are unique because the parasite genome contains four genes that translate into three protein assemblies – destined for the parasite cytoplasm (FRS^{cyto} – a heterodimer), apicoplast and its mitochondria [9,37]. The FRSs couple phenylalanine to the 2-hydroxy group of ribose on the 3′-terminal end of cognate $tRNA^{Phe}$. The three *Pf*-FRSs belong to the class IIc aaRS family and show distinct signatures in their architecture and functional adaptations. The well-studied eukaryotic FRSs generally contain additional editing and DNA-binding domains, whereas the organelle FRSs are monomeric, smaller and lack editing and DNA-binding domains [9,37]. The identification of the highly potent antimalarial compound series of bicyclic azetidines that target the *Pf*-FRS^{cyto} has been a remarkable advancement in antimalarial drug discovery [31]. These lead molecules cure mouse malaria in a single dose and target multiple parasite lifecycle stages, including the transmission stages [31]. The atomic resolution structure of the *Pf*-FRS^{cyto}–drug complex remains unknown; however, availability of drug screening assays and robust high-throughput parasite-based assessment of lead molecules presents a promising scenario.

Case of cytoplasmic lysyl-tRNA synthetase

Within evolutionary lineages, the lysyl-tRNA synthetase (KRS) is the only aaRS that is present as class I and II [44]. Eukaryotes and most prokaryotes contain class II KRSs, whereas some bacteria and archaea contain class I [44]. KRSs from many organisms including *P. falciparum* have been reported to synthesize signaling molecules like diadenosine polyphosphate (Ap4a) that can alter cellular processes like DNA replication, gene expression and ion channel regulation, to mention a few [14,45]. In recent years, the structural and biochemical attributes of *Pf*-KRS^{cyto} have been elucidated in the context of the potent inhibitor CL [15,23,46]. CL inhibits *P. falciparum* growth in blood and liver stages with IC_{50} values below 100 nM [15,23]. The antimalarial effect of CL is highly selective and does not target mammalian cells (or its KRS) [23]. CL fits into the ATP-binding site in *Pf*-KRS^{cyto} and interacts with most of the residues that accommodate the adenosine moiety of ATP [15]. The tetrahydropyran ring is accommodated in a ribose-recognizing subpocket and mimics the ribose moiety of adenosine, whereas the isocoumarin mirrors the adenine [15]. The structural basis for CL selectivity for *P. falciparum* KRS over human KRS has been validated [15,23] and it pivots on two residues at the rims of the ATP-binding pocket (Val328 and Ser344 in *Pf*-KRS^{cyto}); larger versions (Gln and Thr, respectively) are non-accommodating for CL in the *Hs*-KRS active site [15,23]. *Pf*-KRS^{cyto} is currently one of the best-studied models for understanding the CL binding mechanism [15,23], and CL-inspired inhibitors are worthy of further development.

Case of cytoplasmic prolyl-tRNA synthetase

Febrifugine (FF; and its derivatives such as HF) possess very potent antimalarial activity via the inhibition of *P. falciparum* prolyl-tRNA synthetase (*Pf*-PRS^{cyto}) [47]. HF targets blood- and liver-stage malaria parasites with IC_{50} values below 100 nM [13,21,26,27,47]. HF suffers from cytotoxicity issues in the context of human use, but other FF derivatives have shown better selectivity for the parasite in a recent study [26]. Crystal structures of *Hs*-PRS and *Pf*-PRS^{cyto} in complexes with HF + AMPPNP (ATP analog) have been resolved revealing the remarkable basis of HF integration into the PRS active site [13,26,27,47,48]. The piperidine ring of HF competes for L-proline and thus rests in the amino-acid-binding pocket of PRSs, whereas its quinazolinone colonizes the 3′ end of the tRNA (A76) binding pocket [13,26,27,48]. HF and other FF derivatives thus act as dual site inhibitors of *Pf*-PRS^{cyto} and are in contrast to the mode-of-action of CL (discussed earlier). Intriguingly, *Hs*-PRS and *Pf*-PRS^{cyto} contain several unexploited additional sites for drug binding, as revealed by our analysis of at least four subsites in *Pf*PRS^{cyto} based on co-crystal structures of *Hs*-PRS and *Pf*-PRS^{cyto} in complex with different inhibitors (Fig. 4). Thus, *Pf*-PRS^{cyto} represents a highly valued antimalarial target where lead molecules from several different scaffolds have been described – these present opportunities for fine-tuning selectivity and refining their drug-like properties.

Other cytoplasmic *P. falciparum* aminoacyl-tRNA synthetases: atomized yet unexploited

The crystal structures of three other cytoplasmic *Pf*-aaRSs: arginyl-, tryptophanyl- and tyrosyl-tRNA synthetases, have been resolved over the years [10,34,49], although no significant advancement in drugging them has been reported – primarily owing to lack of potent hit compounds that can target these enzymes. Because the recombinant production of each of these enzymes has already been achieved [10,34,49,50], we feel that these *Pf*-aaRSs are ripe for screening in enzyme-based assays using hit compounds identified from phenotypic screenings against *P. falciparum*. Of the 36 *Pf*-aaRSs, the arginyl-tRNA synthetase (*Pf*-RRS^{cyto}) is unique because its enzymatic product (arg-$tRNA^{arg}$) is not only fed into ribosomes for protein translation but arg-$tRNA^{arg}$ can also assist in flagging proteins with arginines destined to be degraded via the N-end rule pathway [49]. Free hemin has the ability to bind and inactivate *Pf*-RRS^{cyto} by driving its dimerization, which structurally occludes $tRNA^{arg}$ binding [49]. The structural basis for hemin–*Pf*-RRS^{cyto} interactions remains elusive, but for drug development the unique structural loops and insertions in *Pf*-RRS^{cyto} over its human counterpart could be exploited (sequence identity between *Hs*/*Pf*-RRSs

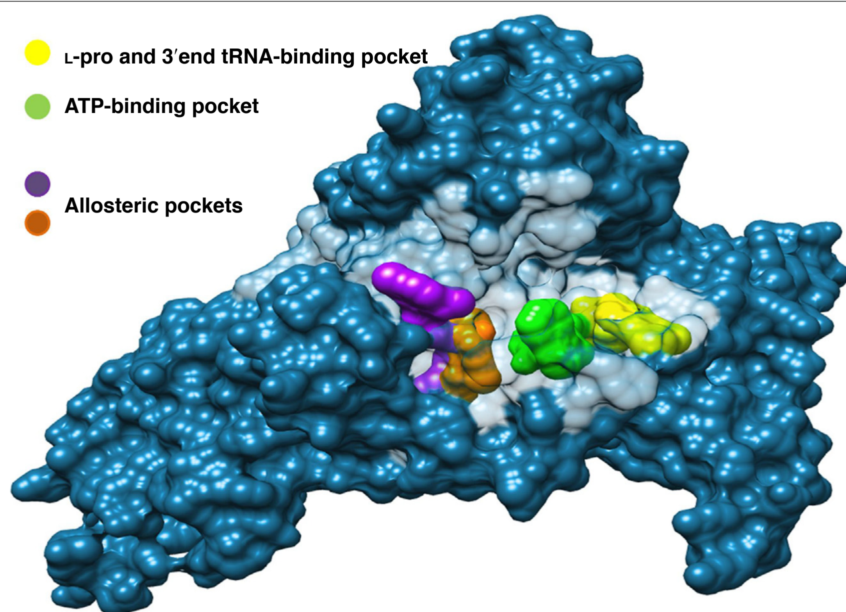

FIGURE 4

Multiple druggable sites in *Pf*-PRS^{cyto}. Surface representation of *Pf*-PRS^{cyto} (PDB ID: 4YDQ) with four different inhibitor molecules: halofuginone (HF, yellow PDB ID: 4YDQ), glyburide (violet, PDB ID: 5IFU), TCMDC-124506 (orange, PDB ID: 4WI1) and modeled Takeda compound B (green, PDB ID: 5VAD). L-Proline and 3′-end tRNA-binding pockets are occupied by halofuginone, the ATP-binding pocket occupied by Takeda compound B and the auxiliary sites are occupied by glyburide and TCMDC-124506.

is ~30%; Fig. 2). For *Pf*-WRS, the crystal structure of the catalytic domain is available [34,50] and the sequence identity between parasite and human enzyme counterparts is moderate ~44% (Fig. 2) [34,50]. A noticeable feature of *Pf*-WRS is the 15-residue insertion in its KMSKS-motif-containing loop; the conformational plasticity of which has been studied earlier [51]. The availability of *Pf*-WRS in its recombinant form will allow screening of inhibitors akin to other *Pf*-aaRSs. Finally, an extensive phenotypic screening project resulted in the shortlisting of >13,000 potent antimalarial compounds, of which two compounds (TCMDC-125870 and TCMDC-141232 with IC_{50} values of ~0.13 and ~0.69 μM, respectively) were predicted to disable the *Pf*-YRS^{cyto} [10,51]. The binding of TCMDC-125870 and TCMDC-141232 to *Pf*-YRS^{cyto} has not been confirmed although, once again because the crystal structure of *Pf*-YRS^{cyto} is available, this project is poised for further interrogation [10]. Similar very elegant work was done on *Pf*-PRS^{cyto} [33]. Hence, it is timely to revisit potent hits from phenotypic screenings to assess their potential to inhibit *Pf*-aaRSs using enzyme-based validation methods, especially in cases like the above three where recombinant protein production protocols have been well established and high-resolution crystal structures are available.

Unexplored pool of *P. falciparum* aminoacyl-tRNA synthetases

There remain a dozen cytoplasmic *Pf*-aaRSs: alanyl-, asparaginyl-, aspartyl-, cysteinyl-, glutamyl-, glutaminyl-, glycyl-, histidinyl-, isoleucyl-, leucyl-, seryl- and valyl-, that need to be explored to identify small molecules capable of high potency *Pf*-aaRS inhibition (Fig. 2). These aaRS enzymes share varying percentages of sequence identity with their human counterparts in the range of ~30–55% (Fig. 2). Among these, benzoxaboroles target the editing site of *Pf*-LRS^{cyto} effectively and the crystal structure for the editing domain of enzymes with this scaffold is known [24,25,32]. However, structural underpinnings of the charging site for *Pf*-LRS^{cyto} remain unknown, and this aaRS could be very valuable to target in conjunction with its editing site inhibitors (i.e., the potential of dual drug binding to the same aaRS). A 13th member: *Pf*-MRS, has been partly studied [17,52], and diarylamine compounds like REP8839 and REP3123, which disable eukaryotic MRSs, have been exploited for parasites like *Trypanosoma brucei* and *P. falciparum* [17,52].

Absence of robust protein production systems and complete crystal structures of the above dozen *Pf*-aaRSs hampers their further exploration – although these aaRSs are of vital importance in the overall thrust at identifying new druggable targets in *P. falciparum*. Hence, one project immediately evident from this present analysis is the pursuit of HTS of potent antimalarial compounds (like the pathogen box hits) against the above-mentioned *Pf*-aaRSs. Each of the cytoplasmic *Pf*-aaRSs listed above has at least three druggable pockets (amino acid, ATP and 3′-end of tRNA-binding sites), notwithstanding additional opportunities like editing subsites and accessory pockets (Fig. 2). It is noteworthy that three subsites offer seven opportunities (i.e., combinations of drug scaffolds) for drug targeting. If the number of subsites per aaRS increases to four (e.g., by the additional occupation of an accessory site) then the combination goes up to 15 druggable sites per aaRS. Those *Pf*-aaRSs that have editing domains in addition to the catalytic ones can be further screened in conjunction with catalytic site inhibitors. Therefore, a rich resource of druggable pockets in aaRSs presents unprecedented possibilities for in terms of identifying drug-like molecules that could target cytoplasmic *Pf*-aaRSs by: (i) blocking multiple sites on the same aaRS with one scaffold

(as exemplified by CL, HF and BN) or multiple inhibitors (potentially by adding ATP-pocket inhibitors along with HF); (ii) blocking multiple *Pf*-aaRSs with the same (or multiple) drug scaffolds (no known example yet). These atypical albeit fully feasible approaches could thus provide very strong foundations for the eventual drugging of an ensemble of aaRS subsites and their corresponding enzymes within *P. falciparum*.

Bipolar *P. falciparum* aminoacyl-tRNA synthetases

Studies from several labs have shown that of the 36 aaRSs in *P falciparum*, four *Pf*-aaRSs (alanyl-, threonyl-, glycyl- and cysteinyl-tRNA synthetase) are single-copy genes that produce dual localized proteins (i.e., shared between cytoplasm and apicoplast) [16,18,38,39]. These four *Pf*-aaRSs, incidentally, also share their first letter amino acid codes with genomic alphabets (ATGC). *P. falciparum* has evolved mechanisms for bipolar targeting of these four *Pf*-aaRSs, and thus achieves a full roster of aaRSs in each of its (three) translational locale [16,18,38,39]. Because *P. falciparum* mitochondria survive by soaking in charged tRNAs from the parasite cytoplasm [37], this implies that alanyl-, threonyl-, glycyl- and cysteinyl-tRNA synthetases supply their charged tRNA products of alanyl-adenylate, threonyl-adenylate, glycyl-aenylate and cysteinyl-adenylate, respectively, to all three translationally independent compartments in *P. falciparum* [18,37,39]. Although the parasite's trick is simple and elegant, this evolutionary solution immediately suggests that drug targeting of any one or more of these four *Pf*-aaRSs (of ATGC) will stall protein synthesis globally for the parasite (mitochondria, cytoplasm and apicoplast). The *Pf*-ATGC-aaRSs therefore present very enticing cases for the screening and discovery of small-molecule inhibitors using HTS methodologies [18,37,39]. From sequence conservation analysis of *Hs* versus *Pf*-ATGC-aaRSs (Fig. 2), it is evident that the percentage identity is in the poor-to-moderate range of ~36–47% (Fig. 2). This lack of high sequence conservation suggests contributions from *Plasmodium*-specific variations in terms of addition or deletion of sequence modules that could be of significance in exploiting these four bipolar *Pf*-ATGC-aaRSs over their human equivalents. In summary, drugging of *Pf*-ATGC-aaRSs thus offers exceptional opportunities for poisoning protein translation in three parasite organelle factories – a tactic that at the outset targets enzymatic activities that are required in multiple locations within the parasite.

Of the four dual-localized *Pf*-aaRSs, encouraging advances have already been achieved for *Pf*-TRS$^{cyto/api}$ in terms of targeting it using the natural product BN (Fig. 3b) [40,53,54]. Despite the lack of crystal structure for *Pf*-TRS$^{cyto/api}$, there is significant understanding of the mode of BN action based on studies from bacterial and human TRSs, which can also be drugged by BN [40]. Bacterial and human TRSs possess four distinct subsites in their architectures that expectedly include pockets for ATP, L-Thr and tRNA but also an additional accessory subsite [40]. Rather remarkably, BN docks to all these four subsites within TRSs, and thus serves as an exemplary example of multi-site targeting within the same aaRS [40]. The potency of BN against *Pf*-TRS$^{cyto/api}$, although structurally unaddressed, presents an avenue for fine-tuning its drug-like properties to enhance selectivity over *Hs*-TRS. The quadripartite druggable regions within *Pf*-TRS$^{cyto/api}$ represent an exceptional opportunity for drug development and exemplify the potential of all *Pf*-aaRSs to be equally targeted.

Concluding remarks

The combined products of aaRS reactions in the form of charged tRNA molecules are an essential biological requirement for viability, because protein synthesis is an indispensable cellular activity. The 36-member *P. falciparum* tRNA synthetase family has been deservedly receiving increasing attention over the past decade as a new source of enzymatic drug targets within the parasite genome. Although many 3D structures of malaria parasite enzymes are now available, the success rate of drugging them remains low owing to lack of potent drug-like molecules that can bind and inactivate these proteins. Additionally, identifying drug-like hits that target novel protein architectures within *P. falciparum* proteins is arduous and expensive. Through the examples we have highlighted here, we show that, despite the seeming handicap of *Pf*-aaRSs (they are evolutionarily conserved as housekeeping enzymes in all cells), they offer tantalizing opportunities for drug development – as long as small-molecule hits can be identified that can differentiate between *Hs* and *Pf*-aaRSs based on subtle sequence and structure variations. Already, cutting-edge structural parasitology and medicinal chemistry studies have resulted in two approved drugs against fungal and bacterial aaRSs: tavaborole (targets LRS, for onychomycosis) and mupirocin (targets IRS, antibacterial). The rapidity with which resistance mutations can arise when aaRSs are targeted needs to be assessed in each case – it is evident that the discovery of new drug scaffolds against *P. falciparum* aaRSs now requires emphasis on phenotypic screening and target-based methods, potentially in parallel. As noted in this work, we are yet to discover potent inhibitors against a dozen *P. falciparum* aaRSs. In addition, all four of the single-gene-copy, bi-localized *Pf*aaRSs (*Pf*-ARS$^{cyto/api}$, *Pf*-TRS$^{cyto/api}$, *Pf*-GRS$^{cyto/api}$ and *Pf*-CRS$^{cyto/api}$) offer striking additional opportunities for drug screening that will have ramifications for protein production across all three translational chambers in malarial parasites. Hence, a sharper focus on this enzyme family is likely to contribute significantly to the (slowly) growing kitty of future antimalarial drugs that the world health community could require in the near future.

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