In silico assessment of natural products and approved drugs as potential inhibitory scaffolds targeting aminoacyl-tRNA synthetases from *Plasmodium*

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Abstract

Malaria remains the leading cause of deaths globally, despite significant advancement towards understanding its epidemiology and availability of multiple therapeutic interventions. Poor efficacy of the approved vaccine, and the rapid emergence of antimalarial drug resistance, warrants an urgent need to expedite the process of development of new lead molecules targeting malaria. Aminoacyl-tRNA synthetases (aaRSs) are essential enzymes crucial for ribosomal protein synthesis and are valid antimalarial targets. This study explores the prospects of (re-)positioning the repertoire of approved drugs and natural products as potential malarial aaRS inhibitors. Molecular docking of these two sets of small-molecules to lysyl-, prolyl-, and tyrosyl- synthetases from *Plasmodium* followed by a comparison of the top-ranking docked compounds against human homologs facilitated identification of promising molecular scaffolds. Raltitrexed and Cefprozil, an anticancer drug and an antibiotic, respectively, showed stronger binding to *Plasmodium* aaRSs compared to human homologs with > 4 kcal/mol difference in the docking scores. Similarly, a difference of ~ 3 kcal/mol in Glide scores was observed for docked Calcipotriol, a drug used for psoriasis treatment, against the two lysyl-tRNA synthetases. Natural products such as Dihydroxanthohumol and Betmidin, having aromatic rings as a substructure, showed preferential docking to the purine binding pocket in *Plasmodium* tyrosyl-tRNA synthetase as evident from the calculated change in binding free energies. We present detailed analyses of the calculated intermolecular interaction for all top-scoring docked poses. Overall, this study provides a compelling foundation to design and develop specific antimalarials.

Keywords Malaria · Drug repurposing · Natural products · Aminoacyl-tRNA synthetases · Virtual screening

Introduction

Malaria continues to kill millions worldwide, with a significant proportion of those infected being children. *Plasmodium falciparum* is the most fatal species accounting for the majority of cases (World malaria report 2019). Over the

years, many anti-malarial compounds have been developed but against most of them like chloroquine, pyrimethamine, and proguanil, resistant strains of the parasite have emerged (Gregson and Plowe 2005). Therefore, an innovative strategy is required to find novel antimalarial compounds. Traditional drug discovery involves de novo identification and validation of new molecular entities (NME), a long and expensive method (Shim and Liu 2014). Worryingly, the average time needed for drug development has increased from 7 to 13 years since the 2000s in the US and EU countries (Pammolli et al. 2011). Drug-repurposing is the identification of new manifestations from existing medicine and its application in the treatment of diseases apart from that drug's intended disease (Pushpakom et al. 2019). This approach can indeed help overcome the initial obstacles in the drug discovery process. A remarkable example of a repurposed drug is the use of sedative thalidomide to treat myeloma. This drug was initially released in the market in 1957 to treat

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nausea in pregnant women, but soon it was found to cause severe congenital disabilities among children with fatal consequences (McBride 1977). After its withdrawal from the market, researchers discovered the anti-tumor activity of this hypnotic drug (D'Amato et al. 1994). Later, the FDA (Food and Drug Administration, USA) approved thalidomide in combination with dexamethasone for myeloma treatment (Ning and Gulley 2010). Though several of these repurposed drugs have been found by serendipity, the method of drug repurposing is gaining popularity with the increasing clinical application. Also, Natural products (NPs) have been a key source of chemical scaffolds in drug development (Firn and Jones 2003). The therapeutic areas of contagious diseases and oncology have gained from extensive diversity of naturally occurring molecular scaffolds, which can interact with many unique targets within the cell, and have been a source of inspiration for most of the FDA-approved drugs for many years (Mishra and Tiwari 2011).

Aminoacyl-tRNA synthetases (aaRSs) are house-keeping enzymes that catalyze the charging of tRNA with their cognate amino acid, a critical step in ribosomal protein synthesis. This enzyme family is structurally well-characterized and, importantly, is a validated antimalarial target (Manickam et al. 2018). A multi-domain architecture with evolutionarily conserved substrate recognition motifs is a salient characteristic of aaRSs (Fig. 1). The Rossman fold domain facilitates ATP and amino-acid binding, while the anticodon binding domain recognizes the tRNA. These enzymes are categorized into two classes based on topology and conserved motifs—Class I has conserved KMSKS and HIGH motifs that mediate ATP binding. In contrast,

Class II enzymes have three conserved sequence motifs 1, 2, and 3 that are crucial for ATP binding (Fig. 1). Several investigations have provided useful insights into the structure–function attributes of the aminoacyl-tRNA synthetase family in *Plasmodium falciparum* (Herman et al. 2015) (Goodman et al. 2016) (Pham et al. 2014). This study aims at evaluating the prospects of approved drugs and natural products to block the substrate-binding pockets of *Plasmodium* aaRSs. The motivation for this study comes from the fact that multiple approved drugs are ATP analogs, e.g., Kinase inhibitors (Bhullar et al. 2018). Some known inhibitors of malarial synthetases indeed have a fused aromatic ring as a substructure (Hoepfner et al. 2012). Natural products also have derivatives of similar organic scaffolds, so it would be insightful to access their binding potential within the synthetase pockets. The medicinal value of natural products is well established—about fifty percent of the marketed drugs are naturally occurring molecules or their derivatives (Newman and Cragg 2012). Importantly, decades of research efforts aimed at the design and development of antimalarials have gained immensely by the exploration of the abundant repertoire of natural products. For instance, artemisinin, which is one of the most effective antimalarials, is an active ingredient of *Artemisia annua*, a herb used in Chinese traditional medicine (Graziose et al. 2010; Wang et al. 2019). These clinically encouraging therapeutic outcomes acclaim natural product scaffolds as the starting point for antimalarial development (Wells 2011). Cladosporin, a fungal secondary metabolite, is a next-generation antimalarial compound that has two vital characteristics as a potential lead for an antimalarial drug. First, it inhibits the growth

Fig. 1 Domain architecture and conserved motifs for the three aminoacyl-tRNA synthetases from *Plasmodium falciparum* showing the Catalytic Domain (CD) and Anticodon Binding Domain (ABD).

Prolyl-tRNA synthetase also has editing domain (EDIT) and C-terminal domain (C_T). The ligands were docked to the substrate binding pockets within the catalytic domain of the three aaRSs

of *Plasmodium* in both blood and liver stages at nanomolar concentration. Second, it shows a stronger and specific inhibition of parasite lysyl-tRNA synthetase as compared to the human homolog (Hoepfner et al. 2012). Despite these essential pharmacological properties, Cladosporin failed as therapeutics against malarial because of its poor oral bioavailability. Nevertheless, this knowledge of a PfKRS specific molecular scaffold was propitious for designing metabolically stable synthetic derivatives (Rusch et al. 2019). The quest for discovering antimalarial compounds from natural sources is far from over. Various types of alkaloids derived from medicinal plants offer privileged scaffolds for antimalarial development (Uzor 2020). Recent in vitro and in vivo studies, using plant extracts from *Artocarpus champeden*, *Callistemon citrinus*, and *Rumex crispus*, show encouraging antimalarial activity (Idris et al. 2019; Larayetan et al. 2019; Widyawaruyanti et al. 2020); opening up avenues for the development of effective therapeutic interventions. It is important to emphasize that there are around 15 known inhibitors, which are secondary metabolites produced by microbes, that target at least eight different microbial aaRSs (Francklyn and Mullen 2019). Most of these inhibitors block the activity of prokaryotic enzymes, while some of these such as cladosporin, borrelidin, and halofuginone (a natural product derivative) specifically inhibit *Plasmodium* lysyl-, threonyl-, and prolyl- tRNA synthetases, respectively (Hoepfner et al. 2012; Jain et al. 2017; Novoa et al. 2014).

The well-established effectiveness of naturally occurring molecular scaffolds against malaria motivated us to research the potential of natural products as malarial aaRS inhibitors systematically. For this research, we have virtually screened two small-molecule libraries against the crystal structures for lysyl-, prolyl-, and tyrosyl- tRNA synthetases.

The catalytic domains from the *Plasmodium* and human homologous pairs are structurally conserved; however, there are important differences in the substrate-binding pockets that have been exploited for structure-based drug design. For instance, Cladosporin discriminates between *Plasmodium* and human lysyl-tRNA synthetase based on the different size of the hydrophobic cavity near the adenine binding pocket. Also, the presence of amino acids with shorter side chains such as Val328 and Ser344 in the PfKRS compared to the corresponding residues in HsKRS contributes to the high specificity of Cladosporin towards PfKRS (Hoepfner et al. 2012). Similar variations in the binding pocket sizes and orientation of the loops around the binding site can be observed in the case of YRS and PRS homologs from the two organisms (Figure S1). Here, we outline a detailed and comprehensive analysis of the differential binding strengths of two sets of compounds with respect to aaRS homologs in humans and *Plasmodium*. The results presented here enabled us to identify potential lead-like molecular scaffolds which would prove useful in the structure-based design of specific antimalarial compounds.

Methods

Preparation of proteins and ligands

We retrieved the crystallographically determined aaRS structures from the Protein Data Bank (PDB) (Berman et al. 2000). All these structures are in holo conformation, i.e., their active site is occupied by either a substrate or a substrate analog (Table 1). We modeled the missing coordinates for the loop residues in PfKRS, HsPRS, and both YRS's

Table 1 Details for the aminoacyl-tRNA structures used in this study. Additional information regarding disordered residues in different aaRSs is given in supplementary Table S1

Organism	aaRS	PDB ID (reference)/resolution (Å)	Bound ligand/binding site	Grid center
<i>Plasmodium falciparum</i>	KRS	4YCV (Fang et al. 2015)/3.41	Cladosporin/ATP binding pocket	Around the bound ligand 'Cladosporin'
	PRS	5IFU (Hewitt et al. 2017)/2.45	Glyburide/amino acid binding pocket	Around bound ligand 'Glyburide'
	YRS	3VGJ (Bhatt et al. 2011)/2.21	Tyrosine/amino acid binding pocket AMP/ATP binding pocket	Specific residues (chain A)—247, 248, 250, 235–238, 206, 207, 209, 210, 60–63, 69–72, 74, 77, 94, 96, 99, 195, 192, 188, 172, 185, 124
<i>Homo sapiens</i>	KRS	4YCU (Fang et al. 2015)/2.10	Cladosporin/ATP binding pocket	Around the bound ligand 'Cladosporin'
	PRS	5VAD (Adachi et al. 2017)/2.36	Proline/amino acid binding pocket 91Y/ATP binding pocket	Specific residues (chain A)—160, 28, 162–167, 27, 41, 31, 52, 56, 283, 284, 280, 288, 281, 515, 706
	YRS	4QBT (Sajish and Schimmel 2014)/2.10	Tyrosine/amino acid binding pocket	Specific residues (chain A)—39–43, 54, 55, 77, 215, 184, 185, 187, 182, 152, 166, 170, 173, 72

by homology derived restraints using Modeller (Sali and Blundell 1993) interface within UCSF Chimera (Pettersen et al. 2004). Detailed information for the missing residues is presented in supplementary table S1. Thus generated full-length structures were energy minimized using the default parameters in the protein preparation wizard of Schrödinger (Madhavi Sastry et al. 2013). We used two different ligand datasets—FDA Approved drugs (2261 molecules) (Wishart et al. 2006) and *Plasmodium falciparum* specific natural compounds from Natural Product Activity and Species source (NPASS) database (2012 molecules) (Zeng et al. 2018). LigPrep wizard was used for generating tautomers as well as the stereochemical and ionization variants for all the molecules. Subsequently, the conformation of all the ligands were energy minimized with optimized potentials for liquid simulations (OPLS_2005) force-field (Jorgensen and Tirado-Rives 1988). QikProp and Ligand Filtering features were used to eliminate compounds that did not follow Lipinski's Rule of Five. We performed all the steps for the preparation of proteins and ligands using Schrödinger software (Schrödinger Release 2019-4: Maestro, Schrödinger, LLC, New York, NY, 2019).

Grid generation and docking

An essential requirement for running docking calculations is to mark the region in which the sampling of the docked poses would be performed. For inhibitor bound aaRS structures, we prepared a grid by selecting the co-crystallized ligand using the default parameters. In the case of substrate-bound aaRSs, the centroid of the active site residues was used to mark the grid center. We used the receptor-grid generation module in Schrödinger to generate input files for docking. Details of the residues constituting the active site in different aaRSs are outlined in Table 1 and Figure S1 has a visual representation of the grid box for all the aaRSs. We docked the two ligand datasets prepared above to all the six aaRSs using the Glide module of Schrödinger (Friesner et al. 2006) in the flexible docking mode, where the program auto-generates different conformation for each input ligand while docking. Glide samples various orientations of the ligand within the grid box and provides a quantitative assessment of the strength of non-bonded interactions between the protein and the ligand through the Glide Score. We have compared the relative strength of ligand binding to each homologous pair of aaRSs using the Prime MM-GBSA module in Schrödinger. This method calculates the change in binding free energy ($\Delta G_{\text{binding}}$) by subtracting the free energy of the components (receptor and ligand) from the free energy of the complex. Energies of each of the three components, i.e., complex, receptor and ligand were calculated using OPLS_2005 force-field and VSGB (Variable dielectric Surface Generalized Born) implicit solvation

model (Bhachoo and Beuming 2017). We have also performed a detailed analysis of the drug-likeness of the top-ranking ligands using SwissADME (Daina et al. 2017). In addition, evolutionary conservation of the ligand interacting aaRS residues was done using ConSurf. PyMol and Python scripts were used to prepare structure and graph figures.

Results and discussion

Repositioning approved drug against malarial aaRSs

We have investigated the plausibility of repositioning the approved drugs and prospects of natural products as potential inhibitors targeting aminoacyl-tRNA synthetases from *Plasmodium falciparum*. To this end, we have performed virtual screening of 3540 molecules (1535 from FDA and 2010 from NPASS) against three pairs of aaRSs from *Plasmodium* and humans. The docking scores for all these protein–ligand dockings are in supplementary file 1. Following is the detailed comparative analysis of the binding profiles of the approved drugs to these aaRS homologs. Interestingly, for each of the three synthetases, we were able to identify a drug molecule that showed preferential binding to malarial aaRS as compared to the human homolog.

Lysyl-tRNA synthetase

For our molecular docking studies with lysyl-tRNA synthetases from *Plasmodium* and humans, we prepared a grid box around the bound Cladosporin in the crystal structures of both aaRSs. Figure 2a shows the distribution of 1273 docked drug molecules based on the Glide Score with the two proteins. Calcipotriol, a side chain analog of Vitamin D₃ hormone calcitriol and originally used to treat psoriasis, showed enhanced interaction with *Pf*KRS as compared to the human homolog. This difference in binding score resulted from Calcipotriol docking to different regions within pockets of the two KRSs (Fig. 2b). The docked pose of Calcipotriol with *Pf*KRS shows that the dihydroxy methylidene cyclohexane moiety of the ligand occupies the ATP-binding pocket like the isocoumarin moiety of Cladosporin and the adenine ring of ATP (Fig. 2c, d) with a docking score of -7.13 kcal/mol. Whereas on analyzing the results in *Hs*KRS, the docking score of Calcipotriol is just -4.19 kcal/mol, and the docked Calcipotriol does not superimpose the inherent Cladosporin (Fig. 2e, f). Importantly, the docking score of the Calcipotriol-*Pf*KRS complex is better than the corresponding score of the complex with the known inhibitor. The Glide score for re-docking Cladosporin to *Pf*KRS crystal structure was -4.21 kcal/mol. Structural analysis of the docked complex shows that the

Fig. 2 **a** Docking scores for the approved drugs with *PfKRS* (PDB ID: 4YCV) and *HsKRS* (PDB ID: 4YCU). Top five ligands based on the Glide score against *PfKRS* are highlighted in blue, while the ligands having the largest difference in scores between the two KRS homologs are red. The details for all the annotated points in the scatterplot are given in Table S2. **b** Superimposition of Calcipotriol poses

in *PfKRS* (yellow) and *HsKRS* (cyan). Our results show a difference of ~3 kcal/mol in the Glide score for these two poses. Superimposition of the docked Calcipotriol with the crystallographic Cladosporin pose (from PDB ID: 4YCV) in the case of *PfKRS* (**c**) and *HsKRS* (**e**) with corresponding intermolecular interactions for the docked pose (**d, f**)

two hydroxyl groups of dihydroxy methylidene cyclohexane part of the docked ligand form hydrogen bonds with some highly conserved residues like side chain of His338 and the backbone of Asn339, in *PfKRS*. These interactions are similar to the Cladosporin-*PfKRS* interactions observed in the crystal structure. In *HsKRS*, Calcipotriol got docked near the surface of the ATP-binding pocket, forming hydrogen bonds with Glu426 (helix α 9) and Arg485 (helix α 10).

Interestingly, the other end of the ligand extends towards the amino acid binding pocket in *PfKRS* but completely misses this pocket when docked with *HsKRS*. The docked pose of Calcipotriol in *PfKRS* is such that it sits just above the backbone of the substrate lysine in the *HsKRS*. A hydrogen bond between the hydroxyl group beside the cyclopropyl ring with the highly conserved residue Glu308 (helix α 7) stabilizes the docked pose. These results show that the docked pose

of Calcipotriol engages both the substrate binding pockets in *Pf*KRS, while it occupies only the ATP binding pocket in case of *Hs*KRS, resulting in the difference of ~ 3 kcal/mol in the docking score of the ligand with respect to the two proteins. The calculated change in binding free energy ($\Delta G_{\text{binding}}$) for the two complexes further substantiates that Calcipotriol shows a stronger binding to *Plasmodium* protein as compared to the human homolog. The $\Delta G_{\text{binding}}$ values for *Plasmodium* and human complexes were -36.4 kcal/mol and -29.2 kcal/mol, respectively.

Prolyl-tRNA synthetase

Prolyl-tRNA synthetase is also a homodimer similar to tyrosyl-tRNA synthetase but belongs to class II of aaRS. We have performed docking using only the monomer and have been able to dock 849 approved drugs to both the homologs (Fig. 3a). In the case of docking with *Pf*PRS, our calculations show Raltitrexed as the top-ranking docked molecule. Raltitrexed inhibits thymidylate synthase and works as a chemotherapeutic drug for colorectal cancer. The docked pose for Raltitrexed in *Pf*PRS superimposed well with the crystallographic pose of Glyburide within the amino acid binding pocket (Fig. 3c, d). Encouragingly, Raltitrexed showed stronger binding to *Pf*PRS compared to Glyburide as evident from their docking scores of -8.48 kcal/mol and -4.09 kcal/mol, respectively. In complex with *Pf*PRS, the carbonyl group of Raltitrexed formed a hydrogen bond with the backbone of Ser33 in the Rossmann fold domain. Also, the carboxylic group adjacent to amine forms a hydrogen bond with the backbone of the highly conserved Asn230. The other end of Raltitrexed, having two carboxylic groups is engaged with the active site via salt-bridge with the side chain of Arg232 (another highly evolutionarily conserved position with *Pf*PRS). Interestingly, these sets of non-bonded interactions between *Pf*PRS and Raltitrexed mimic the binding of Glyburide to the active site of the protein. On the other hand, the docked pose of Raltitrexed within *Hs*PRS, was oriented away from the binding pockets (Fig. 3e, f) and, therefore, had a low docking score of -2.66 kcal/mol. The docked *Hs*PRS-Raltitrexed complex was stabilized by two hydrogen bonds with Ser33 and Lys38, which were formed by two carboxyl groups on one end of Raltitrexed, whereas the other end did not participate in any non-bonded interactions. These differences in intermolecular interactions corroborate well with the quantitative estimates for the strength of Raltitrexed binding to *Pf*PRS and *Hs*PRS having $\Delta G_{\text{binding}}$ values of -52.64 kcal/mol and -20.38 kcal/mol, respectively. Figure 3b shows the superimposed orientations for the docked Raltitrexed against *Plasmodium* and human PRSs. The observed difference in the binding profile of Raltitrexed with these two homologs and the difference of ~ 6 kcal/mol

in the docking scores indicate that this molecule could be a potential lead for antimalarial drug development.

Tyrosyl-tRNA synthetase

Tyrosyl-tRNA synthetase is a class I aaRS and is present as a homodimer with each monomer having binding sites for all the substrates. For our molecular docking studies, we have used only one of the two monomers from the dimeric crystal structure. Using Glide, we could dock 1407 approved drugs to both human and *Plasmodium* YRSs. Figure 4a shows the distribution of these docked molecules based on the Glide Score for docking with the two proteins. Our results show that the top-ranking ligand, i.e., Cefprozil had different docking poses in the binding pockets of human and *Plasmodium* YRS (Fig. 4b). The docked Cefprozil was engaged in multiple non-covalent interactions within the active site of *Pf*YRS. As shown in Fig. 4c, d, the carbonyl group in the ligand participated in hydrogen bond interactions with the residues from both the ATP binding loops YRS. Side Chains of His70 (first histidine of the HIGH loop) and Lys247 (first lysine of the KMSKS loop) were less than $3 \text{ \AA}$ from the ligand's carbonyl functional group. The tyrosyl moiety in Cefprozil showed an analogous set of interactions as observed for the bound tyrosine in the crystal structure. The phenol ring in the ligand was anchored to the active site via bifurcated hydrogen bonding with the side chains of Tyr60 (helix $\alpha 10$) and Asp195 (strand $\beta 2$). Furthermore, the N-atom within the tyrosyl substructure participated in hydrogen bond interactions with backbones of Ile172, Gln192, and Gln210 and with the Glu64 sidechain. Also, the pi-pi and cation-pi interactions with Phe99 (a highly conserved residue in helix $\alpha 5$) and Lys250 (second lysine of the KMSKS loop), respectively, anchored the ligand's aromatic rings within the binding pocket. Interestingly, the docked pose for Cefprozil in the *Hs*YRS active site showed a lesser number of stabilizing non-covalent interactions as compared to the docked pose in *Pf*YRS. We observe that the orientation for the tyrosyl moiety in Cefprozil within *Hs*YRS is markedly different compared to the tyrosine bound in the crystal structure. Analysis of the *Hs*YRS-Cefprozil complex showed only three intermolecular hydrogen bonds (Fig. 4e, f). Docking calculations show that Cefprozil could not access the tyrosine binding pocket in *Hs*YRS and instead got docked near the surface of the active site. Sidechains of Lys81 and Glu148, along with the backbone of Ala147 engaged the ligand around the *Hs*YRS active site. Importantly, neither of the two ATP-binding loops in *Hs*YRS participated in interaction with the docked Cefprozil. Overall, these results indicate that Cefprozil interacts rather weakly within the active site of *Hs*YRS. These observations reconcile well with

Fig. 3 **a** Docking scores for the approved drugs with *Pf*PRS (PDB ID: 5IFU) and *Hs*PRS (PDB ID: 5VAD). Top five high scoring ligands based on the Glide score against *Pf*PRS are highlighted in blue, while the ligands having the largest difference in scores between *Pf*PRS and *Hs*PRS are red. Green triangle represents Raltitrexed, which has the highest docking score with *Pf*PRS (− 8.48 kcal/mol) and has a relatively poor score with *Hs*PRS (− 2.66 kcal/mol). The details for all the

annotated points are given in Table S2. **b** Superimposition of Raltitrexed poses in the *Pf*PRS (magenta) and *Hs*PRS (cyan). Superimposition of the docked Raltitrexed with the crystallographic Glyburide pose (from PDB ID: 5IFU) in the case of *Pf*PRS (c) and *Hs*PRS (e) with corresponding intermolecular interactions for the docked pose (d, f)

the calculated docking scores for Cefprozil to *Pf*YRS and *Hs*YRS, i.e., − 9.34 kcal/mol and − 3.47 kcal/mol, respectively. The difference of ~6 kcal/mol in the docking score with these two YRSs indicates preferential binding to the *Pf*YRS active site compared to *Hs*YRS. Furthermore, we observed a substantial difference in the

calculated $\Delta G_{\text{binding}}$ for Cefprozil binding to *Plasmodium* (− 51.5 kcal/mol) and human (− 19.6 kcal/mol) YRSs. Overall, these quantitative measures demonstrate that Cefprozil could be a potential lead molecule for anti-malarial development.

Fig. 4 **a** Docking scores for the approved drugs with *PfYRS* (PDB ID: 3VGJ) and *HsYRS* (PDB ID: 4QBT). Top five high scoring ligands based on the Glide score against *PfYRS* are highlighted in blue, while the ligands having the largest difference in scores between the two YRS homologs are red. The green triangle represents Cefprozil which has the highest docking score with *PfYRS* (− 9.34 kcal/mol) and a relatively poor score with *HsYRS* (− 3.47 kcal/mol). The

details for all the annotated points are given in Table S2. **b** Superimposition of the docked Cefprozil poses in *PfYRS* (yellow) and *HsYRS* (blue). Superimposition of the docked Cefprozil with the crystallographic substrate pose (from PDB ID: 3VGJ) in the case of *PfYRS* (**c**) and *HsYRS* (**e**) with corresponding intermolecular interactions for the docked pose (**d**, **f**)

Natural products as potential inhibitors of aminoacyl-tRNA synthetases from *Plasmodium*

Natural products constitute a class of organic compounds that are synthesized in living organisms, generally as secondary metabolites. Using a targeted set of compounds from the Natural Products Activity and Species Source (NPASS)

database, we have screened their binding properties against the three pairs of aaRS homologs (Fig. 5). Docking scores for all the compounds against these aaRSs are in supplementary file 1. Details of nonbonded interactions for the top docked poses for different aaRSs are outlined below. Interestingly, our comparative analysis of the dockings demonstrates that at least two compounds showed stronger

Fig. 5 Comparison of the docking scores for natural products with lysyl-, prolyl-, and tyrosyl-tRNA synthetases from *Plasmodium* and humans. The top five molecules, based on the Glide score, against *Plasmodium* are blue, and the molecules with the largest difference in the docking scores with the two homologs are red. In the case of YRS, two of the top five ranking molecules had similar docking

scores resulting in overlap of corresponding blue dots. Green squares represent an overlap of blue and red circles; these are Isocycloheximide, Folic acid, and Dihydroxanthohumol (top), and Betmidin (below) in the case of KRS, PRS, and YRS, respectively. Supplementary Table S3 has additional details for all the annotated points

binding to the *PfYRS* compared to *HsYRS*. Similar comparative analysis for KRS and PRS, however, did not show any outstanding molecule based on the docking scores.

In the case of *PfYRS*, Dihydroxanthohumol showed stronger binding to the active site compared to the binding in *HsYRS*. This compound is isolated from *Humulus lupulus* (Common hop), which belongs to the Cannabaceae or hemp family and is a known inhibitor of Nitric Oxide synthase. The docked Dihydroxanthohumol participated in hydrogen bonds in both amino-acid (Tyr60 or Asp195) and ATP (Tyr60 or Asp195) binding pockets of *PfYRS* (Fig. 6a). The hydroxyphenyl group near C5 of the propane-1-one moiety imitates the binding of Tyrosine in the amino-acid binding pocket. The docked pose superimposed well with the crystallographic pose of the bound amino-acid and AMP. The phenyl group on the other end of propan-1-one, having a dihydroxy and methoxy group, forms hydrogen bonds with Lys247 (the first lysine of KMSKS loop), Asp209, Gly62, and Glu64 (Fig. 6a). Dihydroxanthohumol also engages in pi-pi interaction with Phe99, which is a highly conserved residue in *PfYRS*. In *HsYRS*, Dihydroxanthohumol got docked near the ATP binding pocket but away from the amino-acid binding pocket. Our results show that the hydrogen bonding with the backbone of Gly38, Gly182, and Val212 and Tyr39 side chains stabilized the docked ligand (Fig. 6b). The presence of these multiple non-bonded interactions between the docked Dihydroxanthohumol and *PfYRS* resulted in the Glide score of -9.82 kcal/mol, while for *HsYRS* the score was only -6.35 kcal/mol. The comparison of calculated $\Delta G_{\text{binding}}$ shows a drastic difference in Dihydroxanthohumol interaction with the YRSs. The *Plasmodium* complex had a $\Delta G_{\text{binding}}$ of -72.4 kcal/mol, while for human complex, it was $+29.8$ kcal/mol. These differences the docking scores and calculated $\Delta G_{\text{binding}}$ values

along with the conformational analysis indicates that Dihydroxanthohumol has preferential binding to the malarial YRS as compared to the human homolog. The docked pose for Betmidin (Myricetin 3- α -L-arabinofuranoside) in the *PfYRS* active site forms a bifurcated hydrogen bond with the side chains of Tyr60 and Asp195 (Fig. 6c). Additionally, the docked pose formed a hydrogen bond with the side chain of Tyr188, which is a native interaction between substrate tyrosine and the enzyme, as observed in the crystal structure. Similarly, native contacts of bound AMP in the crystal structure were also observed in the docked pose of Betmidin. The hydroxyl group on the oxolan ring interacts with the backbone of Glu64 and the three hydroxyl groups on trihydroxyphenyl form separate hydrogen bonds with the backbone atoms of Asp61 and Asp209 and to the side chain of His235. These multiple protein-ligand interactions resulted in a docking score of -8.68 kcal/mol. Contrastingly, Betmidin showed a rather weak binding to the *HsYRS* forming just one hydrogen bond with the enzyme, i.e., with the sidechain of Glu148 (Fig. 6d) such that the docking score of Betmidin binding to *HsYRS* had a low value of -0.66 kcal/mol. Our $\Delta G_{\text{binding}}$ calculations accord well with the observed differences in the docking scores. Betmidin-*PfYRS* complex had very strong intermolecular interaction with $\Delta G_{\text{binding}}$ of -50.0 kcal/mol, whereas in case of the Betmidin-*HsYRS* complex the corresponding value was $+67.5$ kcal/mol. This substantial difference in the intermolecular binding energy for Betmidin to the malarial and human YRSs indicates the potential of Betmidin as a privileged molecular scaffold for designing antimalarial compounds. In the case of KRS and PRS, the top-ranking docked ligands in the *Plasmodium* enzyme were Isocycloheximide and Folic acid, respectively. Both these compounds on docking with human aaRSs showed comparable docking scores to the corresponding

Fig. 6 Our results for virtual screening of a natural compounds database against aaRS homologs from *Plasmodium* and humans show Dihydroxanthohumol and Betmidin binds more strongly to the *PfYRS* compared to *HsYRS*. Intermolecular interaction between docked poses of Dihydroxanthohumol and **a** *PfYRS* and **b** *HsYRS*, and Betmidin and **c** *PfYRS* and **d** *HsYRS*. The difference between

the Glide scores for the docked Dihydroxanthohumol and Betmidin against the two homologs was ~3 kcal/mol and ~8 kcal/mol, respectively. Supplementary figure S3 shows intermolecular interactions for top ranking docked poses against KRS and PRS from the two organisms

homologs. Figure S2 shows the intermolecular interactions observed in the top-scoring docked molecules for KRS and PRS. For the top-ranking molecules against the *Pf*aaRSs we have also calculated their drug-likeness using different criteria. We observed that all the small-molecules followed most of these criteria in addition to Lipinski's rule of five (Supplementary Table S3).

Conclusion

The rapid emergence of drug resistance against the existing anti-malarial drugs warrants the need for short-circuiting the rather long and treacherous process of drug discovery. Disrupting *Plasmodium*'s protein biosynthetic machinery is a promising approach to kill the pathogen. Aminoacyl-tRNA synthetases are crucial enzymes for protein translation, and are validated antimalarial targets. Availability of experimentally determined tertiary structures for different aaRSs from *Plasmodium*, and human homologs, foster the avenues for computer-aided discovery of potential lead molecules. We report a meticulous assessment of predicted binding strength

of approved drugs against lysyl-, prolyl-, and tyrosyl- tRNA synthetases. Also, we describe the ranking of a customized subset of natural products in terms of their preferential binding the *Plasmodium* aaRSs. Our in silico results show that drugs such as Calcipotriol, Cefprozil, and Raltitrexed bind strongly and preferentially to the *PfKRS*, *PfPRS*, and *PfYRS*, respectively. Our molecular docking calculations accentuate natural products such as Dihydroxanthohumol and Betmidin that showed stronger binding to *PfYRS* as compared to the corresponding human homolog. Overall, our study underscores specific molecular scaffolds complementary to the active sites within the three *Pf*aaRSs. The outcomes of this study would prove useful in guiding the astute design of potential antimalarial compounds rapidly.

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Author contributions MD ideated the study and designed the methodology. KD and NP performed the experiments. MD, KD, and NP analyzed the results and prepared the figures. KD and NP prepared a

draft for the manuscript which was substantially revised by MD. All the authors approve the submitted manuscript.

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Conflict of interest The authors declare that they have no competing interests.

References

- Adachi R, Okada K, Skene R, Ogawa K, Miwa M, Tsuchinaga K, Ohkubo S, Henta T, Kawamoto T (2017) Discovery of a novel prolyl-tRNA synthetase inhibitor and elucidation of its binding mode to the ATP site in complex with L-proline. *Biochem Biophys Res Commun* 488:393–399. <https://doi.org/10.1016/j.bbrc.2017.05.064>
- Berman HM, Westbrook J, Feng Z, Gilliland G, Bhat TN, Weissig H, Shindyalov IN, Bourne PE (2000) The Protein Data Bank. *Nucleic Acids Res* 28:235–242. <https://doi.org/10.1093/nar/28.1.235>
- Bhachoo J, Beuming T (2017) Investigating protein-peptide interactions using the Schrödinger computational suite. In: Schueler-Furman O, London N (eds) *Modeling peptide-protein interactions: methods and protocols*. Springer, New York, pp 235–254. https://doi.org/10.1007/978-1-4939-6798-8_14
- Bhatt TK, Khan S, Dwivedi VP, Banday MM, Sharma A, Chande A, Camacho N, de Poupiana LR, Wu Y, Craig AG, Mikkonen AT, Maier AG, Yogavel M, Sharma A (2011) Malaria parasite tyrosyl-tRNA synthetase secretion triggers pro-inflammatory responses. *Nat Commun* 2:530
- Bhullar KS, Lagarón NO, McGowan EM, Parmar I, Jha A, Hubbard BP, Rupasinghe HPV (2018) Kinase-targeted cancer therapies: progress, challenges and future directions. *Mol Cancer* 17:48. <https://doi.org/10.1186/s12943-018-0804-2>
- D'Amato RJ, Loughnan MS, Flynn E, Folkman J (1994) Thalidomide is an inhibitor of angiogenesis. *Proc Natl Acad Sci USA* 91:4082–4085. <https://doi.org/10.1073/pnas.91.9.4082>
- Daina A, Michielin O, Zoete V (2017) SwissADME: a free web tool to evaluate pharmacokinetics, drug-likeness and medicinal chemistry friendliness of small molecules. *Sci Rep* 7:42717
- Fang P, Han H, Wang J, Chen K, Chen X, Guo M (2015) Structural basis for specific inhibition of tRNA synthetase by an ATP competitive inhibitor. *Chem Biol* 22:734–744. <https://doi.org/10.1016/j.chembiol.2015.05.007>
- Firn RD, Jones CG (2003) Natural products—a simple model to explain chemical diversity. *Nat Prod Rep* 20:382–391
- Francklyn CS, Mullen P (2019) Progress and challenges in aminoacyl-tRNA synthetase-based therapeutics. *J Biol Chem* 294:5365–5385. <https://doi.org/10.1074/jbc.REV118.002956>
- Friesner RA, Murphy RB, Repasky MP, Frye LL, Greenwood JR, Halgren TA, Sanschagrin PC, Mainz DT (2006) Extra precision glide: docking and scoring incorporating a model of hydrophobic enclosure for protein–ligand complexes. *J Med Chem* 49:6177–6196. <https://doi.org/10.1021/jm051256o>
- Goodman CD, Pasaje CFA, Kennedy K, McFadden GI, Ralph SA (2016) Targeting protein translation in organelles of the Apicomplexa. *Trends Parasitol* 32:953–965. <https://doi.org/10.1016/j.pt.2016.09.011>
- Graziose R, Lila MA, Raskin I (2010) Merging traditional Chinese medicine with modern drug discovery technologies to find novel drugs and functional foods. *Curr Drug Discov Technol* 7:2–12. <https://doi.org/10.2174/157016310791162767>
- Gregson A, Plowe CV (2005) Mechanisms of resistance of malaria parasites to antifolates. *Pharmacol Rev* 57:117–145. <https://doi.org/10.1124/pr.57.1.4>
- Herman JD, Pepper LR, Cortese JF, Estiu G, Galinsky K, Zuzarte-Luis V, Derbyshire ER, Ribacke U, Lukens AK, Santos SA, Patel V, Clish CB, Sullivan WJ, Zhou H, Bopp SE, Schimmel P, Lindquist S, Clardy J, Mota MM, Keller TL, Whitman M, Wiest O, Wirth DF, Mazitschek R (2015) The cytoplasmic prolyl-tRNA synthetase of the malaria parasite is a dual-stage target for drug development. *Sci Transl Med* 7:288ra77. <https://doi.org/10.1126/scitranslmed.aaa3575>
- Hewitt SN, Dranow DM, Horst BG, Abendroth JA, Forte B, Hallyburton I, Jansen C, Baragaña B, Choi R, Rivas KL, Hulverson MA, Dumais M, Edwards TE, Lorimer DD, Fairlamb AH, Gray DW, Read KD, Lehane AM, Kirk K, Myler PJ, Wernimont A, Walpole C, Stacy R, Barrett LK, Gilbert IH, Van Voorhis WC (2017) Biochemical and structural characterization of selective allosteric inhibitors of the plasmodium falciparum drug target, Prolyl-tRNA-synthetase. *ACS Infect Dis* 3:34–44. <https://doi.org/10.1021/acsinfecdis.6b00078>
- Hoepfner D, McNamara CW, Lim CS, Studer C, Riedl R, Aust T, McCormack SL, Plouffe DM, Meister S, Schuierer S, Plikat U, Hartmann N, Staedtler F, Cotesta S, Schmitt EK, Petersen F, Supek F, Glynn RJ, Tallarico JA, Porter JA, Fishman MC, Bodenreider C, Diagana TT, Movva NR, Winzler EA (2012) Selective and specific inhibition of the *Plasmodium falciparum* Lysyl-tRNA synthetase by the fungal secondary metabolite cladosporin. *Cell Host Microbe* 11:654–663. <https://doi.org/10.1016/j.chom.2012.04.015>
- Idris OA, Wintola OA, Afolayan AJ (2019) Evaluation of the bioactivities of *Rumex crispus* L. leaves and root extracts using toxicity, antimicrobial, and antiparasitic assays. *Evid Based Complement Alternat Med* 2019:1–13. <https://doi.org/10.1155/2019/6825297>
- Jain V, Yogavel M, Kikuchi H, Oshima Y, Hariguchi N, Matsumoto M, Goel P, Touquet B, Juman RS, Tacchini-Cottier F, Harlos K, Huston CD, Hakimi M-A, Sharma A (2017) Targeting prolyl-tRNA synthetase to accelerate drug discovery against malaria, leishmaniasis, toxoplasmosis, cryptosporidiosis, and coccidiosis. *Structure* 25:1495–1505. <https://doi.org/10.1016/j.str.2017.07.015>
- Jorgensen WL, Tirado-Rives J (1988) The OPLS [optimized potentials for liquid simulations] potential functions for proteins, energy minimizations for crystals of cyclic peptides and crambin. *J Am Chem Soc* 110:1657–1666. <https://doi.org/10.1021/ja00214a001>
- Larayetan R, Ololade ZS, Ogunmola OO, Ladokun A (2019) Phytochemical constituents, antioxidant, cytotoxicity, antimicrobial, antitrypanosomal, and antimalarial potentials of the crude extracts of *Callistemon citrinus*. *Evid Based Complement Alternat Med* 2019:1–14. <https://doi.org/10.1155/2019/5410923>
- Madhavi Sastry G, Adzhigirey M, Day T, Annabhimoju R, Sherman W (2013) Protein and ligand preparation: parameters, protocols, and influence on virtual screening enrichments. *J Comput Aided Mol Des* 27:221–234. <https://doi.org/10.1007/s10822-013-9644-8>
- Manickam Y, Chaturvedi R, Babbar P, Malhotra N, Jain V, Sharma A (2018) Drug targeting of one or more aminoacyl-tRNA synthetase in the malaria parasite *Plasmodium falciparum*. *Drug Discov Today* 23:1233–1240. <https://doi.org/10.1016/j.drudis.2018.01.050>
- McBride WG (1977) Thalidomide embryopathy. *Teratology* 16:79–82. <https://doi.org/10.1002/tera.1420160113>
- Mishra BB, Tiwari VK (2011) Natural products: an evolving role in future drug discovery. *Eur J Med Chem* 46:4769–4807. <https://doi.org/10.1016/j.ejmech.2011.07.057>
- Newman DJ, Cragg GM (2012) Natural products as sources of new drugs over the 30 years from 1981 to 2010. *J Nat Prod* 75:311–335. <https://doi.org/10.1021/np200906s>

- Ning YM, Gulley JL (2010) Phase II trial of bevacizumab, thalidomide, docetaxel, and prednisone in patients with metastatic castration-resistant prostate cancer. <https://www.ncbi.nlm.nih.gov/pubmed/20308663>. Accessed 8 Jan 2019
- Novoa EM, Camacho N, Tor A, Wilkinson B, Moss S, Marín-García P, Azcárate IG, Bautista JM, Mirando AC, Francklyn CS, Varon S, Royo M, Cortés A, Ribas de Pouplana L (2014) Analogs of natural aminoacyl-tRNA synthetase inhibitors clear malaria in vivo. *Proc Natl Acad Sci USA* 111:E5508–5517. <https://doi.org/10.1073/pnas.1405994111>
- Pammolli F, Magazzini L, Riccaboni M (2011) The productivity crisis in pharmaceutical R&D. *Nat Rev Drug Discov* 10:428–438. <https://doi.org/10.1038/nrd3405>
- Pettersen EF, Goddard TD, Huang CC, Couch GS, Greenblatt DM, Meng EC, Ferrin TE (2004) UCSF Chimera—a visualization system for exploratory research and analysis. *J Comput Chem* 25:1605–1612. <https://doi.org/10.1002/jcc.20084>
- Pham JS, Dawson KL, Jackson KE, Lim EE, Pasaje CFA, Turner KEC, Ralph SA (2014) Aminoacyl-tRNA synthetases as drug targets in eukaryotic parasites. *Int J Parasitol Drugs Drug Resist* 4:1–13. <https://doi.org/10.1016/j.ijpddr.2013.10.001>
- Pushpakom S, Iorio F, Eyers PA, Escott KJ, Hopper S, Wells A, Doig A, Williams T, Latimer J, McNamee C, Norris A, Sanseau P, Cavalla D, Pirmohamed M (2019) Drug repurposing: progress, challenges and recommendations. *Nat Rev Drug Discov* 18:41–58. <https://doi.org/10.1038/nrd.2018.168>
- Rusch M, Thevenon A, Hoepfner D, Aust T, Studer C, Patoor M, Rollin P, Livendahl M, Ranieri B, Schmitt E, Spanka C, Gademann K, Bouchez LC (2019) Design and synthesis of metabolically stable tRNA synthetase inhibitors derived from cladosporin. *Chem-biochem Eur J Chem Biol* 20:644–649. <https://doi.org/10.1002/cbic.201800587>
- Sajish M, Schimmel P (2014) A human tRNA synthetase is a potent PARP1-activating effector target for resveratrol. *Nature* 519:370–373. <https://doi.org/10.2210/pdb4qbt/pdb>
- Sali A, Blundell TL (1993) Comparative protein modelling by satisfaction of spatial restraints. *J Mol Biol* 234:779–815. <https://doi.org/10.1006/jmbi.1993.1626>
- Shim JS, Liu JO (2014) Recent advances in drug repositioning for the discovery of new anticancer drugs. *Int J Biol Sci* 10:654–663. <https://doi.org/10.7150/ijbs.9224>
- Uzor PF (2020) Alkaloids from plants with antimalarial activity: a review of recent studies. *Evid Based Complement Alternat Med* 2020:1–17. <https://doi.org/10.1155/2020/8749083>
- Wang J, Xu C, Wong YK, Li Y, Liao F, Jiang T, Tu Y (2019) Artemisinin, the magic drug discovered from traditional Chinese medicine. *Engineering* 5:32–39. <https://doi.org/10.1016/j.eng.2018.11.011>
- Wells TNC (2011) Natural products as starting points for future anti-malarial therapies: going back to our roots? *Malar J* 10(Suppl 1):S3. <https://doi.org/10.1186/1475-2875-10-S1-S3>
- Widyawaruyanti A, Harwiningtias N, Tumewu L, Hafid AF, Soetjipto, (2020) Effect of formulated *Artocarpus champeden* extract on parasite growth and immune response of *Plasmodium berghei* — infected mice. *Evid Based Complement Alternat Med* 2020:1–7. <https://doi.org/10.1155/2020/4678634>
- Wishart DS, Knox C, Guo AC, Shrivastava S, Hassanali M, Stothard P, Chang Z, Woolsey J (2006) DrugBank: a comprehensive resource for in silico drug discovery and exploration. *Nucleic Acids Res* 34:D668–672. <https://doi.org/10.1093/nar/gkj067>
- World malaria report (2019) <https://www.who.int/publications-detail/world-malaria-report-2019>. Accessed Jan 18 2020
- Zeng X, Zhang P, He W, Qin C, Chen S, Tao L, Wang Y, Tan Y, Gao D, Wang B, Chen Z, Chen W, Jiang YY, Chen YZ (2018) NPASS: natural product activity and species source database for natural product research, discovery and tool development. *Nucleic Acids Res* 46:D1217–D1222. <https://doi.org/10.1093/nar/gkx1026>