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The antimalarial Mefloquine targets the *Plasmodium falciparum* 80S ribosome to inhibit protein synthesis

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Abstract

Malaria control is heavily dependent on chemotherapeutic agents for disease prevention and drug treatment. Defining the mechanism of action for licensed drugs, for which no target is characterized, is critical to the development of their second-generation derivatives to improve drug potency towards inhibition of their molecular targets. Mefloquine is a widely used antimalarial without a known mode of action. Here, we demonstrate that mefloquine is a protein synthesis inhibitor. We solved a 3.2 Å electron cryo-microscopy structure of the *Plasmodium falciparum* 80S-ribosome with the (+)-mefloquine enantiomer bound to the ribosome GTPase-associated center. Mutagenesis of mefloquine-binding residues generates parasites with increased resistance, confirming the parasite-killing mechanism. Furthermore, structure-guided derivatives with an altered piperidine group, predicted to improve binding, show enhanced parasitocidal effect. These data reveal one possible mode of action for mefloquine and demonstrate the vast potential of cryo-EM to guide the development of mefloquine derivatives to inhibit parasite protein synthesis.

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Malaria is a major protozoan parasitic disease that inflicts an enormous burden on global human health. In 2015 the disease resulted in an estimated 429,000 deaths with several hundreds of millions of people infected 1. The causative agents of malaria are a group of protozoan parasites that belong to the genus *Plasmodium*, a member of the ancient apicomplexan phylum of vertebrate pathogens, with *P. falciparum* and *P. vivax* being responsible for the majority of disease mortality and morbidity, respectively 2.

Antimalarial chemotherapies have long been the gold-standard utility for the prevention and treatment of malaria. Over many decades, many different classes of antimalarials have been clinically approved and deployed as frontline treatments to combat malaria 3. Despite the long-standing usage of these drugs, their mode of actions in mediating parasite killing are not well defined. Mefloquine (MFQ) has been one of the most effective antimalarials since it was first developed and has been used as a chemoprophylactic drug by visitors staying in malaria endemic areas. Neurological side effects associated with MFQ usage 4, have often precluded drug being used widely as a first choice for preventative treatment. MFQ has, however, been used in combination with the front line antimalarial drug artemisinin globally to treat malaria, constituting one of the many classes of artemisinin-combination therapies (ACT) pivotal to malaria control. Importantly, in the context of regions that have prevalent pools of artemisinin resistant parasites, recent reports have shown that artemisinin-resistant strains of *P. falciparum* are sensitive to MFQ due to decreasing copy number of *Pfmdr1*, a marker of mefloquine resistance 5. In an urgent response to stem the spread of ACT resistant parasites beyond the province of western Cambodia, the World Health Organization (WHO) has recommended the re-introduction of artesunate and MFQ combination therapy in those regions to combat multi-drugs resistant strains of *P. falciparum* 6. Conversely, in regions where MFQ resistance is prevalent, dihydroartemisinin-piperaquine treatment is preferentially deployed. Despite the major role of MFQ in malaria prevention and its utility in controlling resistant parasites to other ACTs, the molecular basis for its mode of action is, however, not known. Previous studies have suggested that the molecular target(s) for MFQ likely resides in the parasite cytoplasm since efflux of MFQ from the cytoplasm to the parasite food vacuole by the *Pfmdr1* encoded drug transporter Pgh-1, is the predominant mechanism of MFQ resistance 7–10. Furthermore, a large-scale screen of antimalarial drugs previously implied that MFQ might be a putative inhibitor of the *P. falciparum* cytoplasmic ribosome 11. To this end, defining the mode of action of MFQ in the malaria parasite along with high-resolution structural elucidation of the drug bound to its target would enable structure-guided development of second-generation mefloquine derivatives to enhance drug inhibition on the molecular target(s).

Here, we demonstrate that MFQ mediates killing of the malaria parasite by inhibition of parasite protein synthesis through direct binding to the cytoplasmic ribosome (Pf80S) of *P. falciparum*. We have solved the cryo-EM structure of the Pf80S ribosome in complex with MFQ at 3.2 Å resolution, revealing the interaction between the (+)-MFQ enantiomer with residues within the GTPase-associated center of the Pf80S ribosome. The mechanism of parasite killing by MFQ via Pf80S is confirmed by genetic interrogation of key binding residues, with transgenic parasites possessing amino acid substitutions predicted to alter MFQ binding showing enhanced resistance to the drug. Furthermore, using the high-resolution cryo-EM structure as a reference, we have been able to design *de novo* MFQ

derivatives with modifications to a critical MFQ piperidine group. The synthesis of such drugs demonstrated that these MFQ derivatives have enhanced antimalarial activity correlating with structure-activity relationship. Collectively, these data establish the Pf80S ribosome as one of the molecular target(s) of MFQ-mediate parasite killing. Our cryo-EM structure of the Pf80S-MFQ complex serves as an important reference for the design of new MFQ-based derivatives, expanding our tools to inhibit parasite protein synthesis.

MFQ inhibits cytosolic protein synthesis in *P. falciparum*

We first determined the half maximum inhibitory concentration (EC_{50}) of MFQ mediated killing of the 3D7 strain of *P. falciparum*, which showed potent antimalarial activity with an EC_{50} of 25.3 nM (Table 1). The effect of MFQ on translation activity was then tested using incorporation of radiolabelled S₃₅-methionine and S₃₅-cysteine as reporters for protein synthesis. MFQ inhibited protein synthesis by 55%, while parasites cultured in the presence of a non-translation inhibitory compound chloroquine (CQ) showed no inhibition (Fig. 1a). MFQ-mediated translation inhibition was, however, weaker than other, highly toxic cytosolic translation inhibitors such as cycloheximide (CHX) (90%) and emetine (68%) (Fig. 1a). Parasites incubated with doxycycline (DOX), a translation inhibitor that is believed to target only the ribosome of the parasite plastid (apicoplast) organelle 12, 13, showed no effect on cytosolic translation (Fig. 1a). No obvious parasite morphological changes following drug treatment were observed with parasites treated with various antimalarials (CQ, CHX, DOX, EME, MFQ and QUI) showing intact mitochondria and nucleus (see Supplementary Data Fig. S1), indicating the assay conditions did not result in significant non-specific cytotoxicity. Collectively, these results support the hypothesis that MFQ is an inhibitor of cytosolic translation.

Cryo-EM structure of the Pf80S-MFQ complex at 3.2 Å resolution

To demonstrate that MFQ directly acts on the parasite ribosome, the core machinery responsible for *P. falciparum* cytosolic protein synthesis, we solved the structure of *P. falciparum* cytoplasmic ribosome (Pf80S) in the presence of MFQ racemic mixture by cryo-EM at an overall resolution of 3.2 Å (Supplementary Data Table S1, Fig. S2a-c). A difference map calculated between this reconstruction and Pf80S in its apo-form 14 showed two independent continuous densities with shape and size congruent with MFQ when visualized at a threshold of 5 standard deviations (Supplementary Fig. 3a). The well-resolved densities enabled the accurate placement of two MFQ molecules (Fig. 2a-b, Supplementary Fig. 3). The primary MFQ binding-site (designated based on location and correlation with MFQ tolerance, as described below) was located within the GTPase-associated center (GAC) of the large ribosomal subunit (Pf60S) (Fig. 2c and 2e), comprised from the protein uL13, the sarcin-ricin loop of uL6, ribosomal RNA helices 94-5 and expansion segment (ES) 13, where this site interacted with a (+) enantiomer of the MFQ molecule ((+)-MFQ). This region is critical for translation, coordinating the elongation steps of protein synthesis by binding the translational GTPases, and activating the energy dependent translocation of the tRNA-mRNA complex through the ribosome 15, 16. A secondary binding site was located at the peripheral surface of the Pf60S subunit (Supplementary Fig. 3b) where this site interacted with a (-) enantiomer of the MFQ

molecule. Two residues (Tyr290 and His294) from uL4 form a pocket that accommodates the quinoline ring of MFQ at this secondary site (Supplementary Fig. 3b). In *P. vivax*, however, His294 is substituted by Ser294 in this distal MFQ binding site (Supplementary Fig. 3c). Since *P. vivax* is sensitive to MFQ, divergence in this secondary binding site does not, as such, correlate with the inhibitory activity of MFQ on *P. falciparum* and *P. vivax*. Furthermore, since this region has no known role in translation, we believe it is unlikely that MFQ binding at such a site could impact protein synthesis. Of note, the observation of primary (functional) and secondary (likely physiologically irrelevant) binding sites for the antibiotic tetracycline have similarly been reported previously¹⁷.

The identified primary MFQ binding site lies within a crevice formed by a helix of the ribosomal protein uL13 (residues 45 – 59) and ES13 of 28S rRNA (Fig. 2d and 3a). The non-polar residues Leu15 and Ile42 of uL13 interact with the hydrophobic trifluoromethyl group (CF₃) located on C₈ of the quinoline ring (Fig. 3a). On the opposite end of the quinoline ring, the 2-CF₃ group forms a hydrophobic interaction with two aromatic residues (Tyr53 and Phe56) of uL13 (Fig. 3a). The quinoline ring is further stabilized through a cation- π interaction with a magnesium ion coordinated to the backbone phosphate of base C1442 (Fig. 3a). The hydroxyl group of the linker that bridges the quinoline and piperidine ring forms a hydrogen bond with the phosphate backbone of base G1441 (Fig. 3a). Finally, the secondary amine group of the piperidine ring forms a further hydrogen bond with Glu55 of uL13 (Fig. 3a). The inter-atomic distances between (+)-MFQ and interacting residues are within the range of 2.6 – 3.5 Å (Fig. 3b). The nature of this binding site is consistent with structure-activity studies when MFQ was originally conceived¹⁸. Thus, all three functional moieties of (+)-MFQ (quinoline, piperidine ring and the hydroxyl linker) are required for binding to the GAC, while a combination of hydrophobic and hydrogen bonds form the basis of the interaction. To our knowledge, this site of the eukaryotic 80S ribosome represents a novel binding site for a translation inhibitor. Although the thiopeptide and orthosomycin classes of antibiotics also target the GAC, they interfere directly with the binding of elongation factors to the ribosome (Supplementary Fig. 4)¹⁹. However, given that MFQ and thiopeptide/orthosomycin each target the GAC, this suggests that MFQ functions to inhibit parasite protein synthesis by inhibiting the polypeptide elongation step.

MFQ binding residues of protein uL13 of the Pf80S confer functional parasite killing by MFQ

To assess if the primary MFQ binding site in uL13 is the site of action for MFQ-mediated parasite killing, targeted mutagenesis was conducted using CRISPR genome editing technology²⁰. Amino acid substitutions (Leu15Ser and Ile42Ser) were introduced into uL13 in 3D7 parasites (Supplementary Fig. 5, Table S2-3), while wild type residues (Leu15 and Ile42) were introduced as a positive control. In the control experiment, parasites were obtained within two weeks under drug selection using the dihydrofolate reductase inhibitor, WR99210, however no parasites were recovered in two separate experiments when attempts were made to introduce both Leu15Ser and Ile42Ser. This implies that Ser substitutions of Leu15 and Ile42 in uL13 disrupt the function of GAC of the Pf80S ribosome, leading to parasite death. These data demonstrate the essentiality of the GAC for parasite viability,

suggesting (+)-MFQ binding to this site may contribute to parasite death. Consequently, we introduced single substitutions to the MFQ binding pocket (Ile42Ala, Glu55Ala, Phe56Ala, Leu140Phe) creating four transgenic parasite lines. All four transgenic parasite lines carrying single substitutions were recovered in two weeks under WR99210 drug selection. MFQ sensitivity testing of each were compared to control parasites carrying an integrated wild type uL13 gene to test the significance of the MFQ uL13 binding pocket in parasite killing. Despite numerous attempts to purify pure (+) and (-) enantiomers of MFQ with different methods, purification of the MFQ chiral enantiomers for drug sensitivity testing was not possible for this study. As a result, we performed MFQ sensitivity testing using a racemic mixture. Transgenic parasites carrying each single amino acid substitution were more resistant to MFQ, which gave EC₅₀s 1.4 – 1.7 fold higher (36.6 nM – 43.8 nM) than control parasites transfectant for the wild type allele (EC₅₀ = 26 nM) (Fig. 3c, Table 1, *P* values < 0.05). These data confirm the primary MFQ binding pocket in PfL13 of the 80S ribosome contributes to MFQ-mediated parasite killing.

Previous studies have demonstrated that sexual stages of *P. falciparum* are insensitive to MFQ21, 22. During this phase, the parasite switches to variant forms of rRNA that together with the ribosomal proteins form an S-type ribosome that is distinct from the A-type ribosomes found in asexual stages23. Comparison of the RNA sequence of the MFQ binding pocket between rRNA variants reveals a single base deletion within ES13 of the S-type ribosome (C1440 deletion, A-type numbering) (Fig. 3d). Such a change is expected to disrupt the local conformation of the primary MFQ binding pocket thus potentially explaining the resistance of gametocytes to MFQ. Finally, structural conservation of the new binding pocket was explored to determine if it was predictive of tolerance of other major protozoan parasites to MFQ. Each of the binding elements are strictly conserved between *P. vivax* and *P. falciparum* (Fig. 3e), which is consistent with the MFQ sensitivity of *P. vivax*24. *Trypanosoma brucei* is also sensitive to MFQ25, and most of the MFQ-binding elements are identical except for a conservative Glu55Gln substitution (Fig. 3e) that would preserve the hydrogen bond formed with the NH group on the piperidine ring of MFQ. In contrast, *Toxoplasma gondii*, which is insensitive to MFQ26, has a non-conservative Glu55Arg substitution that would be predicted to sterically hinder binding of the piperidine ring moiety (Fig. 3e).

Cryo-EM structure based design of MFQ derivatives with enhanced antimalarial activity

Our high-resolution cryo-EM structure of the Pf80S-MFQ complex serves as a reference-guide to develop MFQ derivatives with improved potency towards inhibition of the Pf80S ribosome. Comparative structural analysis with the human cytosolic ribosome27 revealed two non-conservative substitutions in uL13 found within the MFQ binding pocket (Supplementary Fig. 6a). The first substituted residue (Glu55Ala; *Pf*: human) would eliminate the hydrogen bond between the NH group of the piperidine ring and uL13 (Fig. 3a and Supplementary Fig. 6a). As already shown, when a Glu55Ala substitution is performed in *P. falciparum*, it leads to lower MFQ binding and thus a higher EC₅₀ as expected (Table 1). The second substituted residue (Leu59Lys) would sterically inhibit the binding of MFQ

by clashing with C₄ of the piperidine ring (Fig. 1b, 3a and Supplementary Fig. 6). This suggested that this site could be exploited to enhance drug affinity towards the *P. falciparum* 80S ribosome. Furthermore, substantial structural differences in the MFQ binding pocket of the human mitochondrial ribosome²⁸ indicates that MFQ should not be able to inhibit human mitochondrial protein synthesis (Supplementary Fig. 6b).

As a proof of concept towards this goal, we designed derivatives of MFQ possessing hydrophobic groups that would extend into the parasite-specific Leu59 region of the binding pocket (Fig. 4a-d, Supplementary Data Table 4), while maintaining hydrogen bond interactions with Glu55 and the G1441 nucleotide. Synthesis of these MFQ derivatives (following previously described methods^{29, 30}) and evaluation against *P. falciparum* parasites in culture demonstrated subset of these derivatives (MFQ_D3-5) showed 1.9 – 2.4 fold enhancement in potency towards parasite killing (Table 1: $P < 0.05$). Thus, changes in parasite inhibitory potency of MFQ derivatives was found to be entirely consistent with interaction of MFQ to the PfuL13 binding pocket.

Discussion

Understanding the mode of actions of clinically used antimalarial drugs is important for designing new compound derivatives that can potentially improve inhibition of their molecular targets. To this end, at least two critical pieces of information are required to achieve this goal – first is the identification of the molecular targets inhibited by these drugs, and an equally important second step, is the high-resolution structure of the drug bound to the molecular target to enable structure-guided drug design to improve the potency of the drug for target inhibition. Here we have solved these problems for the antimalarial MFQ by revealing the *P. falciparum* 80S ribosome as one of the targets of MFQ-mediated parasite killing. In addition, a high-resolution cryo-EM structure of the Pf80S with bound (+)-MFQ enantiomer presented in this study, along with proof of principle synthesizing of MFQ derivatives with enhanced antimalarial activity, this body of work establishes the foundation for designing new MFQ derivatives to inhibit parasite protein synthesis

The inhibition of Pf80S by MFQ is consistent with the known site of action of MFQ being in the parasite cytoplasm. This has been demonstrated previously that the removal of MFQ from the parasite cytoplasm into the food vacuole by the drug transporter Pgh-1 is the predominate basis for MFQ resistance^{7–10}. Furthermore, the mechanism of MFQ resistance in *P. falciparum* is inversely correlated with chloroquine (CQ) resistance⁸, suggesting that the primary mode of action of MFQ is not in the parasite food vacuole, the compartment where CQ acts to inhibit heme polymerization. By solving the structure of MFQ bound to the Pf80S, this has led to the identification of binding residues in the 28S ribosomal RNA and protein PfuL13 that interact with the (+)-MFQ enantiomer. This site is part of the GAC of the eukaryotic ribosome known for its important role in the polypeptide elongation step during protein synthesis, suggesting this is the stage that (+)-MFQ inhibits. Importantly, CRISPR-cas9-mediated amino acid substitution of (+)-MFQ binding residues in uL13 generated transgenic parasites with increased resistance (highest EC₅₀ = 43.8 nM: Table 1) to the drug. The measured EC₅₀s of these transgenic parasites (36.6 – 43.8 nM) in response to MFQ treatment is within the range of published EC₅₀s measured in field isolates

(mostly clustered within 35 - 60 nM) that have a MFQ resistance profile 31. It is important to note that the mechanism of MFQ resistance mediated by Pgh-1 in *P. falciparum* is independent of the molecular target of the drug (the mode of killing), which is the aim of investigation in this study. Based on genetics evidence from four independent single amino acid substitutions of PfuL13 with significantly higher resistance to MFQ, (Fig 3c and Table 1), the data demonstrate that the Pf80S ribosome is one of the target of MFQ-mediated parasite killing.

Interestingly, the potency of MFQ in inhibiting parasite protein synthesis (55%) is relatively lower than the highly toxic translation inhibitor CHX (90 %) (Fig. 1a). Mechanistically, CHX and MFQ work distinctly based on the mode of their interactions with the ribosome. CHX competitively blocks the binding of deacylated tRNA to the E-site of the 60S subunit 32, whilst on the contrary, binding of (+)-MFQ to its primary binding site in the PfuL13 pocket of the GAC does not directly compete for binding with any factors. This difference in the binding mode likely explains the variation in translation inhibition potency of the two translation inhibitors with radically distinct modes of interaction with the 60S subunit. Similar to other antibiotics such as thiopeptide and orthosomycin, (+)-MFQ also binds to the GAC of Pf60S subunit. Although thiopeptide/orthosomycin utilize a different binding site within the GAC compared to (+)-MFQ19, nevertheless, (+)-MFQ would similarly be expected to function by inhibiting the polypeptide elongation step during parasite protein synthesis. Furthermore, we have demonstrated the functional importance of the PfuL13 MFQ pocket by introducing amino acid substitutions to residues that form this pocket. By replacing Leu15 and Ile42 with Ser, this resulted in a lethal phenotype after transfection, indicating the essential nature of this pocket of the GAC for generating viable parasites, implying an essential function of the GAC for protein synthesis. Single amino acid substitutions to the PfuL13 pocket with the resultant change in EC50s in response to MFQ treatment also demonstrate the functional importance of this site of the GAC.

The nature of (+)-MFQ binding to the primary binding site is dominated by a number of hydrophobic residues of PfuL13 that form this pocket (Leu15, Ile42, Tyr53, Phe56, Leu59, Leu140), whilst a charge residue (Glu55) of PfuL13 and the sugar phosphate backbone of the 28S ribosomal RNA (G1441, C1442) also contribute to (+)-MFQ binding. Importantly, comparison of uL13 between the human and *P. falciparum* 80S ribosomes reveals significant differences exist in the MFQ pocket. This observation provides a foundation for improving the potency of MFQ towards better inhibition of parasite protein synthesis. Two divergent residues in human and parasite uL13 (Glu55Ala and Leu59Lys *Pf*: human) are readily identifiable (Supplementary Fig. 6a), which form the basis for increasing the potency of (+)-MFQ on the parasite translation machinery. We have shown by introducing Glu55Ala that mimics the human ribosome increased the EC50 of this transgenic parasite from 26 nM to 38.3 nM compared to isogenic wild type control. We hypothesize that by an iterative optimization process, (+)-MFQ derivatives that effectively engage with Glu55 and Leu59 of the *P. falciparum* uL13 pocket may generate more potent compounds that inhibit parasite protein synthesis (Fig 4 and Table 1). Together with recent structural analyses of the Pf80S 14, 33 showing the many parasite specific features along with structural dynamics unique to the parasite ribosome, these data reinforce the idea that the *P. falciparum* 80S ribosome is an increasingly attractive target for antimalarial drug development. Although improving the

potency of (+)-MFQ towards inhibition of parasite protein synthesis is important for the improvement of on-target inhibition, other factors such as safety concerns with MFQ-associated neurological toxicity due to off-target effects will also need to be overcome in order to develop second generation MFQ derivatives with clear clinical benefit over the parental form. Furthermore, noting that a 90 nM of MFQ (IC₉₀) concentration only inhibited translation by 55 % (Fig. 1a), this suggests other unidentified targets are likely to be inhibited by MFQ racemic mixture. Since the cryo-EM structure of the Pf80S-MFQ complex presented in this study shows the (+) form of MFQ enantiomer bound to the GTPase-associated center-PfL13 pocket (Fig 2-3), this suggests that the (-) form of MFQ enantiomer may be a key factor inhibiting other molecular targets in the parasite.

The identification of MFQ as a protein synthesis inhibitor raises the question of whether other related antimalarials such as quinine (QN) and lumefantrine (LF) may also inhibit parasite protein synthesis through the PfL13 pocket. Although these compounds have a related chemical scaffold, the substantial alterations in their structure would argue against their ability to interact with the PfL13 MFQ pocket. Further biochemical characterization of QN and LF would be required to determine their effect on parasite protein synthesis.

Finally, in this study we have demonstrated how cryo-EM can function as an attractive tool for the development of MFQ-based improved protein-synthesis inhibitors. The low yield of Pf80S using cultured parasites has so far precluded the ability to crystalize the Pf80S for structural studies of drug interaction, although sufficient ribosome material may now be feasible for use in biological assays 34. Together with recent elucidation of the structure of the Pf80S-emetine complex 14, the barrier to structure determination based on crystallisation is effectively removed. Thus cryo-EM is now the method of choice for the design of new inhibitors to the Pf80S ribosome.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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Fig. 1. MFQ inhibits cytosolic translation in *P. falciparum*.

a, Translation inhibitory activity of antimalarial compounds, cycloheximide (CHX) at 1.3 μ M, doxycycline (DOX) at 17 μ M, chloroquine (CQ) at 110 nM, emetine (EME) at 105 nM and mefloquine (MFQ) at 90 nM. Asterisks indicate significant differences observed compared to CQ treatment. Mean $\pm$ SD are shown. Each assay was undertaken in triplicate of four independent occasions. **b**, Chemical structure of MFQ.

Fig. 2. MFQ interacts with the GTPase-associated center of the *P. falciparum* large ribosomal subunit.

a-b, Cryo-EM density map of the primary MFQ binding pocket in the absence (**a**) and presence of MFQ (**b**). MFQ is represented as yellow sticks and binding residues are in purple. Oxygen is in red, nitrogen in blue, fluorine in cyan, and magnesium in green. **c,** Magnified EM density of (+)-MFQ depicted in various orientations. **d,** Ribosomal protein PfuL13 and rRNA ES13 form the MFQ binding pocket. Hydrophobic residues are colored in gray. Structure is derived from 112,347 particles from 829 micrographs (see Supplementary

information). **e**, Atomic model of the Pf80S-MFQ complex is shown from the A-site entry side. Magnified inset shows the composition of the GTPase-associated center (GAC) with bound MFQ.

Fig. 3. The primary binding site for (+)-MFQ.

a, Amino acid residues from the protein PfuL13 and bases from ES13 of the 28S rRNA involved in binding to (+)-MFQ. **b**, Residues that interact with (+)-MFQ with inter-atomic distances indicated. **c**, Mefloquine-mediated growth inhibition of control *P. falciparum* parasites carrying an integrated, wild type copy of uL13 gene and four transgenic parasite lines carrying single amino acid substitutions at the uL13 MFQ binding pocket. Data are shown as the mean $\pm$ SD of three biological replicates with each biological replicate representing three experimental replicates. **d**, Divergence in the ES13 part of the MFQ

binding pocket between the *P. falciparum* A type (blood stage) and S type (sexual stage) ribosomes. A single nucleotide C1440 in the A-type 28S rRNA is deleted in the S-type 28S rRNA. MFQ binding residues are highlighted in a box. **e**, Sequence alignment of uL13 from *P. falciparum*, *P. vivax*, *T. gondii* and *T. brucei*. Residues involved in binding to MFQ are highlighted with asterisks.

Fig. 4. Structure based design of MFQ-derivatives.

a, Chemical structure of MFQ_D1. **b**, MFQ_D1 docked into the MFQ binding pocket. **c**, Chemical structure of MFQ_D2. **d**, MFQ_D2 docked into the MFQ pocket. **e**, Parasite growth inhibition assay measuring the inhibitory activity of MFQ derivatives on 3D7 parasites. Data are shown as the mean $\pm$ SD of three biological replicates with each biological replicate representing three experimental replicates.

Table 1
Antimalarial activity of mefloquine and derivatives.

Drug	Strain tested	EC ₅₀ (nM)	P value
MFQ	3D7	25.3 ± 3.4	-
MFQ	3D7.transgenic Wild Type uL13	26.0 ± 1.2	-
MFQ	3D7.uL13.I42A	39.9 ± 5.6	0.0314^a
MFQ	3D7.uL13.E55A	38.3 ± 4.0	0.0218^a
MFQ	3D7.uL13.F56A	43.8 ± 6.4	0.0243^a
MFQ	3D7.uL13.L140F	36.6 ± 5.3	0.0442^a
MFQ_D1	3D7	12.4 ± 4.5	0.0588 ^b
MFQ_D2	3D7	40.9 ± 10.8	0.0771 ^b
MFQ_D3	3D7	11.1 ± 1.1	0.0251^b
MFQ_D4	3D7	13.3 ± 2.4	0.0433^b
MFQ_D5	3D7	10.6 ± 3.6	0.0367^b
MFQ_D6	3D7	15.8 ± 2.3	0.0671 ^b
MFQ_D7	3D7	15.6 ± 2.7	0.0676 ^b

^aComparisons of EC₅₀ of transgenic parasites with L13 variants to transgenic parasite with wild type L13.

^bComparisons of EC₅₀ of 3D7 parasites treated with MFQ-derivatives to MFQ control.

Boldface indicates a statistically significant difference indicated by *P*-values calculated by the t-test.