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New leads for drug repurposing against malaria

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Malaria is threatening a resurgence because of drug resistance against frontline artemisinin-based combination therapies (ACTs). This necessitates the development of alternate routes for malaria treatment. Here, we present a refined focus on US Food and Drug Administration (FDA)-approved over-the-counter (OTC) drugs that could be repurposed. We analyzed growth inhibition data for *Plasmodium falciparum* and *Plasmodium berghei* in the context of 189 and 37 drugs (total of 226), respectively. Of these, our analyses revealed 18 currently used drugs that would be suitable for further development as potential antimalarials. Eight identified drugs share enzymatic targets between the human host and the malaria parasite, providing a platform for mechanistic and drug selectivity studies that could provide optimized leads as next-generation antimalarials.

Introduction

Malaria, a life-threatening infectious disease caused by protozoan parasites of the genus *Plasmodium*, is transmitted by female *Anopheles* mosquitoes [1]. In humans, five species of *Plasmodium* (*P. falciparum*, *P. vivax*, *P. malariae*, *P. ovale* and *P. knowlesi*) cause malaria, with *P. falciparum* and *P. vivax* posing the biggest threats [1,2]. The World Malaria Report in 2017 estimated 216 million cases of malaria with ~445,000 fatalities in 2016 alone [2]. Immunocompromised sections of the population, such as infants, children under the age of 5, pregnant women and those with viral infections are most susceptible to malaria infection and death [2]. Thus, malaria remains a public health threat and the discovery of new antimalarials is an urgent necessity.

A major challenge faced in malaria treatment is the development of parasitic resistance towards antimalarials [3]. First discovered in 1934, chloroquine quickly proved a highly successful antimalarial [4]. However, resistance to chloroquine appeared as early as 1957, and was observed in Colombia and other parts of South-East Asia (SEA) [4]. Similarly, strains of *P. falciparum* resistant to artemisinin-based combination therapy (ACT), the most widely recommended treatment for malaria, were reported in SEA in 2009 [5]. These outcomes emphasize the constant need for newer lead molecules in the antimalarial drug pipeline. Drug repurposing presents itself as a viable option to counter malaria, particularly in the context of over-the-counter (OTC), human use-approved medication. Here, we analyzed previously published parasite growth inhibition data for both *P. falciparum* and the murine parasite *Plasmodium berghei* in the context of 189 and 37 drugs (total of 226), respectively [6,7]. Our analyses reveal 18 human drugs that appear suitable for repurposing, with eight drugs having enzymatic targets that are shared between hosts and parasites. Given that malaria poses a serious threat to infants and pregnant women, we also focused on the safety of the drugs short-listed above in these high-risk groups. Eleven of the 18 drugs were found to be suitable for pediatric prescription. These ranged from drugs that are safe for infants aged 14 days and older (lopinavir), to drugs safe for children above the age of 6 years (amlodipine besylate and clemastine fumarate). Fifteen of the 18 short-listed drugs belong to FDA categories B and C, thus making them a safe option for pregnant women if their benefits outweigh potential risks. Of the 18 short-listed drugs, the proton pump inhibitors (PPIs) esomeprazole magnesium and omeprazole require urgent focus as they are not only safe for both high-risk groups, but are also orally administered OTC drugs whose enzyme targets in humans are shared with the *Plasmodium* parasite. Conservation in enzyme targets between the host and parasite provides a ready platform

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for structure–function studies that can dissect the enzyme–drug interactions to address issues of potency, drug selectivity and drug resistance [8–11].

Collation of data from FDA-approved drugs

To identify potential drug candidates (PDCs) for repurposing, we analyzed 226 molecules previously screened by two groups [6,7]. Of these drugs, 189 had been assessed by Chong *et al*. and showed >50% inhibition of blood-stage *P. falciparum* at 10 μM, whereas the other 37 drugs had been analyzed by Derbyshire *et al*. in a liver-stage *P. berghei* model [6,7]. We applied an IC_{50} cut-off range of ~2.5 μM to the FDA-approved drugs in our list of 226 and short-listed 18 candidates (Fig. 1). The 18 short-listed candidates were further categorized based on multiparametric drug criteria, including their safety profiles in pregnant women and infants. Thirteen of the 18 short-listed candidates had been tested against blood-stage *P. falciparum* [6]. The other five (of 18) PDCs had been tested against liver-stage *P. berghei* [7] (Fig. 1). Of the 18 short-listed PDCs, four (clemastine fumarate, loperamide hydrochloride, omeprazole and esomeprazole magnesium) are OTC drugs, whereas the remaining 14 can be obtained via prescription only (Fig. 2). The four OTC drugs are of particular interest because they are more easily obtainable by patients in high-risk regions. Two of these drugs had been tested against blood-stage *P. falciparum* (clemastine fumarate and loperamide hydrochloride), whereas the other two had been tested against liver-stage *P. berghei* (omeprazole and esomeprazole magnesium) (Fig. 1). The IC_{50} values for these OTC drugs ranged from clemastine fumarate at 2.2 μM, to esomeprazole magnesium at 0.29 μM (Fig. 2). Both omeprazole and esomeprazole magnesium had IC_{50} values <1 μM, making them potential choices for repurposing and/or development as antimalarials. All four OTC drugs are orally administered (Fig. 2).

Of the 14 prescription PDCs (Fig. 2), 11 had been tested against blood-stage *P. falciparum* cultures (verteporfin, amlodipine besylate, triamterene, rocuronium bromide, azithromycin, auranofin, ritonavir, lopinavir, raloxifene hydrochloride, gramicidin A, and pentamidine isethionate), whereas the other three (clomiphene citrate, telmisartan, and cyclosporin A) had been tested against liver-stage *P. berghei* (Fig. 1) [6,7]. The prescription-only PDCs ranged in IC_{50} values from verteporfin at 2.8 μM, to cyclosporin A at 1.7 nM [6,7]. Six of these drugs (raloxifene hydrochloride, gramicidin A, pentamidine isethionate, clomiphene citrate, telmisartan and cyclosporin A) had IC_{50} values <1 μM (Fig. 2) [6,7]. In terms of their routes of administration, 11 of the 14 prescription PDCs are either injectable, oral or topically administered (Fig. 2). The remaining three prescription PDCs (azithromycin, pentamidine isethionate and cyclosporin A) can be administered through multiple routes. Azithromycin, an azalide antibiotic, can be administered orally (tablet, capsule, or solution), topically (eye drops) or intravenously (Fig. 2). Pentamidine isethionate, an antiprotozoal most commonly used to treat African trypanosomiasis, is administered either as an intravenous or intramuscular injection, or as an inhalable powder (Fig. 2). Cyclosporin A, an immunosuppressant drug, can be administered orally (capsule or solution), topically (ophthalmic emulsion), or intravenously (Fig. 2).

In the context of potency of PDCs, seven drugs with the lowest IC_{50} values are cyclosporin A (1.7 nM), telmisartan (25 nM), clomiphene citrate (0.22 μM), esomeprazole magnesium (0.29 μM),

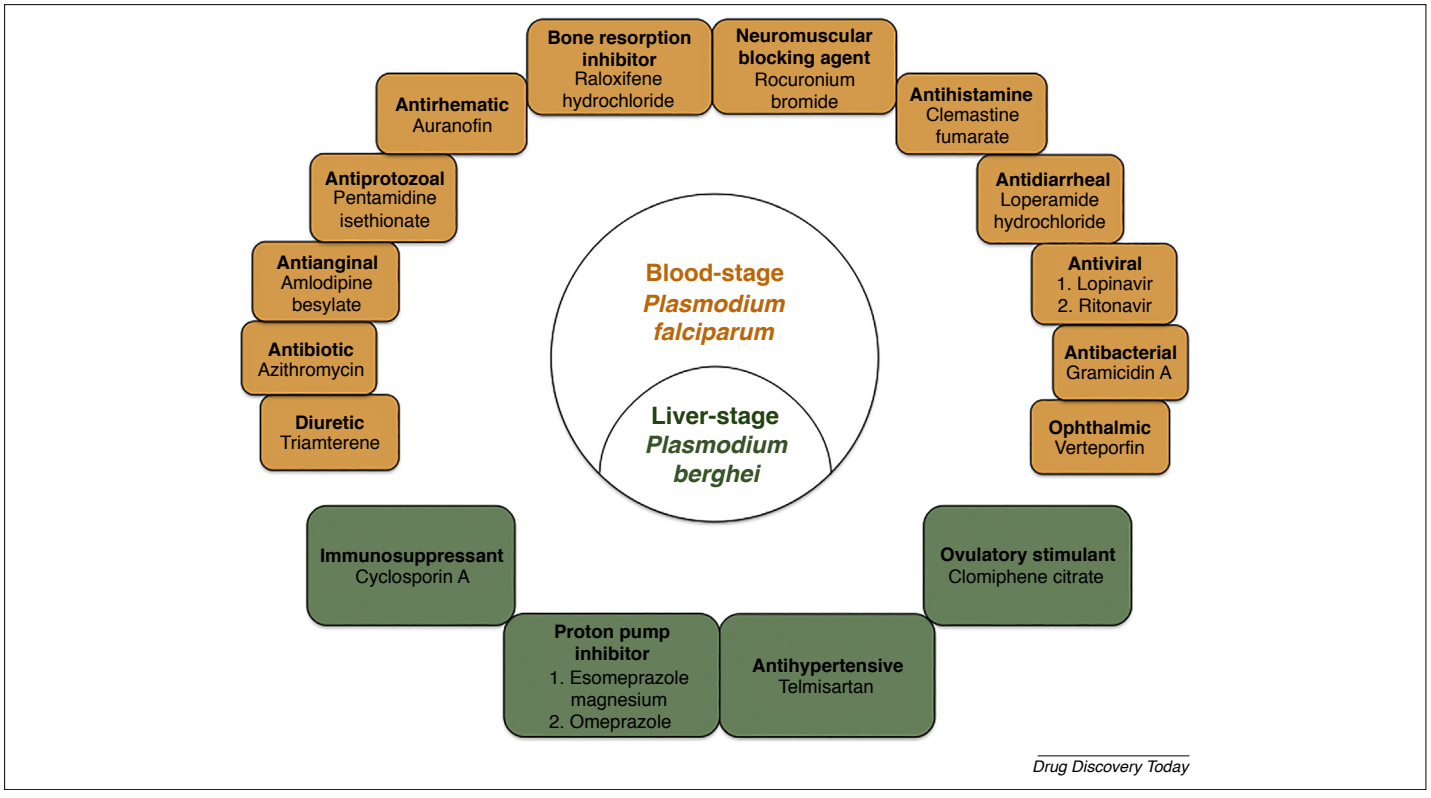


FIGURE 1

Drug classes of potential drug candidates (PDCs). The 18 identified PDCs and their corresponding drug classes are shown [6,7]. The drugs tested against blood-stage *Plasmodium falciparum* (orange) and liver-stage *Plasmodium berghei* (green) are marked.

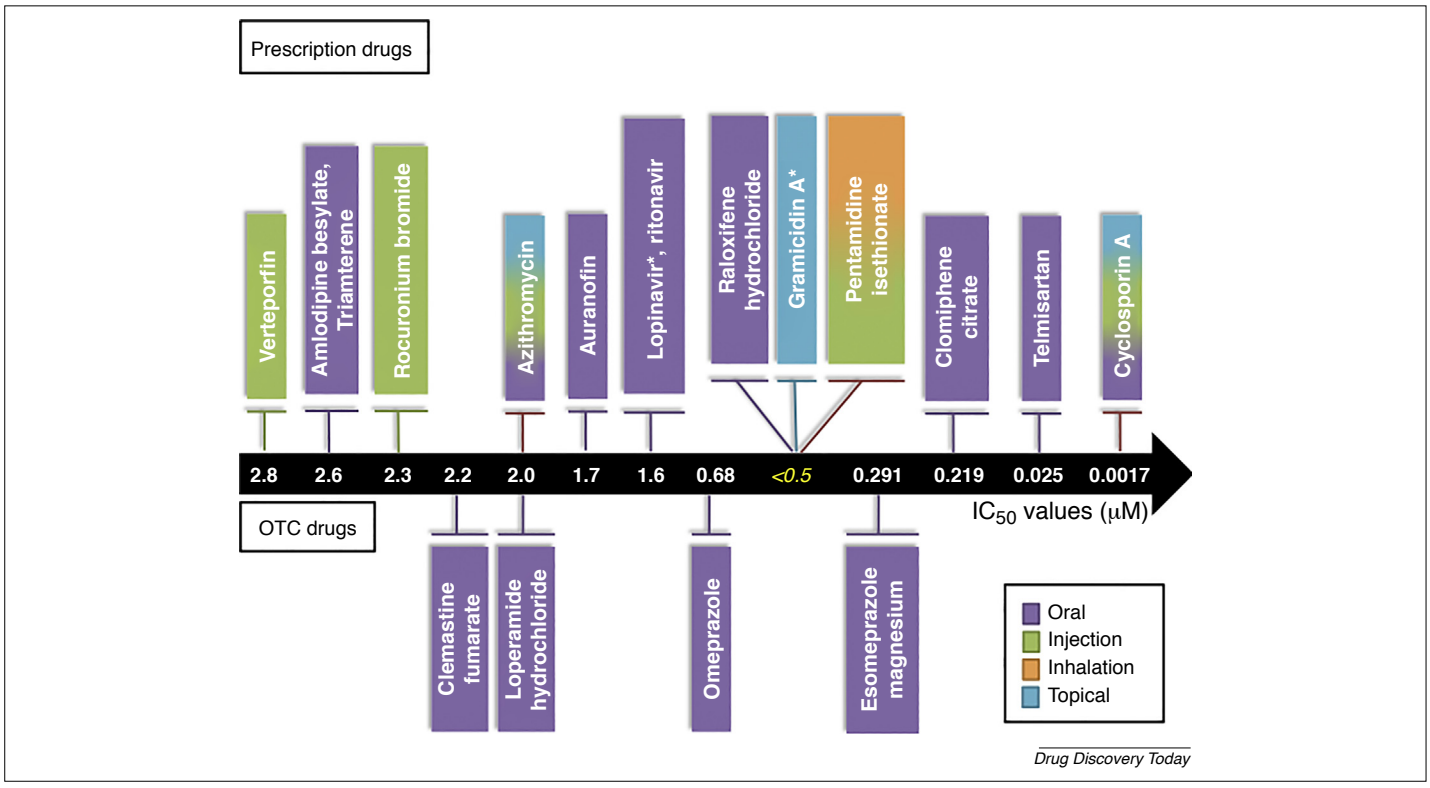


FIGURE 2

Preliminary classification of potential drug candidates (PDCs). The 18 PDCs are shown in decreasing order of their IC_{50} values (μM) [6,7]. All prescription PDCs are listed on top, while over-the-counter (OTC) drugs are listed below the arrow representing IC_{50} values. The PDCs are also color-coded based on their routes of administration: oral (purple), injectable (green), inhalable (orange) and topical (blue). Asterisks indicate PDCs whose US Food and Drug Administration (FDA)-approved forms are combination drugs that include not only the compound tested against the malarial parasites *in vitro* but also other active ingredients.

raloxifene hydrochloride (<0.5 μM), gramicidin A (<0.5 μM) and pentamidine isethionate (<0.5 μM) (Fig. 2). In the case of gramicidin A, the drug tested against blood-stage human *P. falciparum* was gramicidin A alone, whereas the FDA-approved form was a combination drug containing gramicidin (a heterogenous mixture of gramicidin A, B, and C), neomycin sulfate and polymyxin B sulfate (used primarily to treat eye infections). Similarly, the FDA-approved form of lopinavir is a combination drug also containing ritonavir, whereas the drug tested against blood-stage *P. falciparum* was lopinavir alone [6].

The 18 PDCs studied here have different advantages of their own (Fig. 1). Cyclosporin A, telmisartan, clomiphene citrate, esomeprazole magnesium, raloxifene hydrochloride, gramicidin A, pentamidine isethionate and omeprazole have IC_{50} values <1 μM and are more potent compared with the remainder, whereas clemastine fumarate, loperamide hydrochloride, omeprazole and esomeprazole magnesium are more easily accessible because they are OTC drugs. For efficacy trials in combination with existing antimalarial therapies such as artemisinin and chloroquine, the four OTC drugs (clemastine fumarate, loperamide hydrochloride, esomeprazole magnesium, and omeprazole) could be assessed immediately for cases where easy access is more crucial than potency. Two of these OTC drugs (omeprazole and esomeprazole magnesium) have IC_{50} values between 250–680 nM, flagging them as suitable candidates that could provide starting points for optimizing their drug-like properties in the context of potency, selectivity and resistance (Fig. 2).

Repurposable drugs for high-risk groups

Pregnant women and children below the age of 5 are most susceptible to infection and death resulting from malaria [2]. We assessed the safety profiles of the 18 short-listed PDCs in pediatric patients as well as pregnant women (https://dailymed.nlm.nih.gov/), as discussed below.

Pediatric use

To ensure that the PDCs screened and proposed in this study are safe for pediatric prescription, we classified them based on the minimum age at which they can be prescribed to pediatric patients (Fig. 3). For each of these drugs, an assessment of the relative benefits to patient groups versus adverse effects was analyzed. Of the 18 PDCs, 11 are suitable for pediatric use. These ranged from drugs that can be prescribed to infants 14 days and older, to drugs that can be prescribed only to children 6 years and older (Fig. 3). Lopinavir, in combination with ritonavir, is prescribed to infants 14 days and older to treat HIV-1. However, it can also cause adverse effects such as cardiac toxicity, renal failure and respiratory issues in preterm neonates. Its 42% ethanol content must also be noted before administering this drug to infants. Ritonavir by itself is prescribed only to infants over 1 month of age with HIV. This protease inhibitor can also cause adverse reactions such as anemia, neutropenia, hyperamylasemia and thrombocytopenia in >3% of pediatric patients. Esomeprazole magnesium is used to treat patients older than 1 month of age with short-term erosive esophagitis resulting from gastroesophageal reflux disease (GERD). This

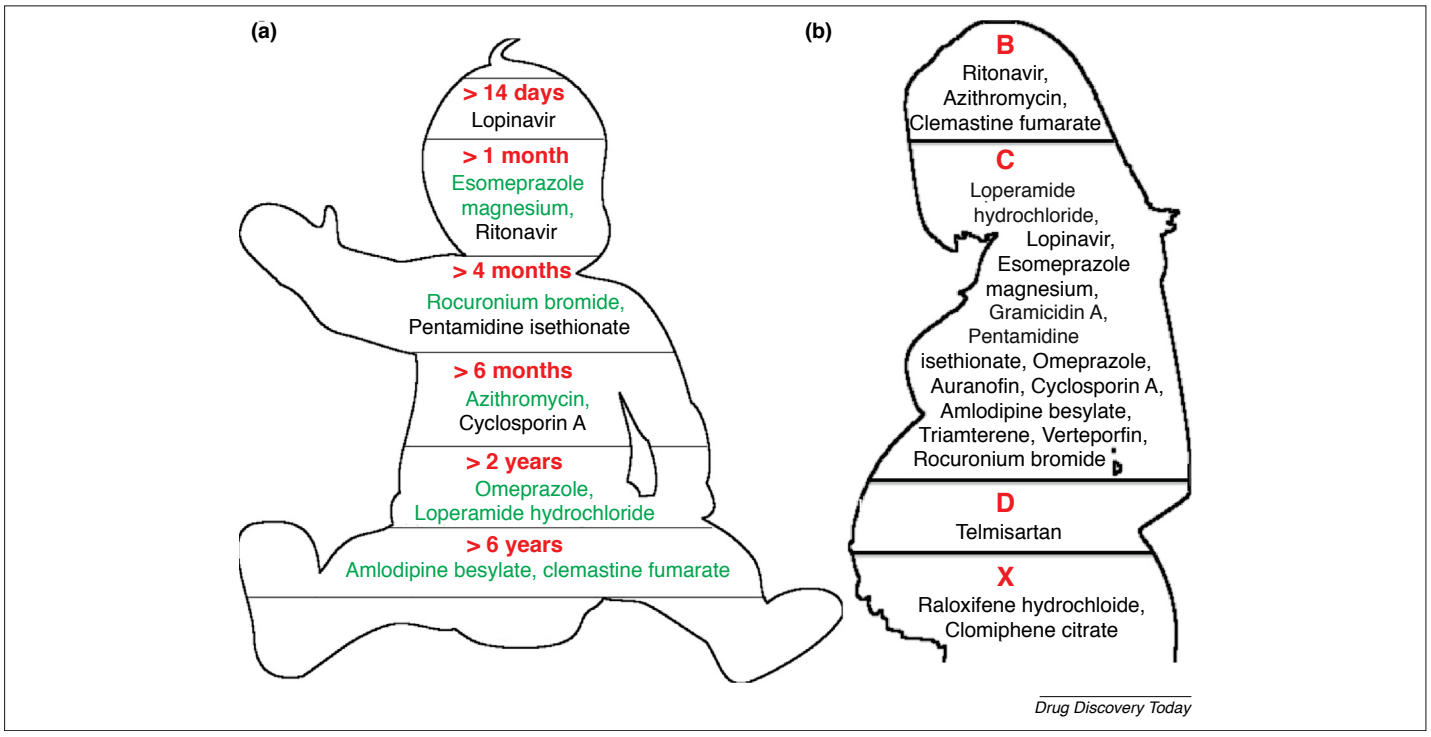


FIGURE 3

Suitability of potential drug candidates (PDCs) in high-risk groups. (A) The 18 PDCs categorized based on the minimum age required for prescription in pediatric patients. Safe drugs with no serious adverse effects in infants and children are highlighted in green. (B) The 18 PDCs categorized based on US Food and Drug Administration (FDA) pregnancy categories B, C, D, and X.

PDC is a better option for pediatric administration because its adverse effects during clinical trials in children mainly included diarrhea, headaches, abdominal pain, regurgitation and somnolence. Rocuronium bromide and pentamidine isethionate are both suitable for use in infants above the age of 4 months. Rocuronium bromide is a neuromuscular blocking agent administered to infants who are 3 months or older, during intubation, with no serious adverse effects observed. Tachycardia and other arrhythmias were seen only in <1% of patients during clinical trials, and the only major adverse effect was anaphylaxis in patients with high sensitivities to this drug. The safety of intravenous and intramuscular pentamidine isethionate has been established in immunocompromised pediatric patients older than 4 months of age to treat *Pneumocystis carinii* pneumonia. However, the adverse effects of this PDC during clinical trials included nephrotoxicity, hypotension, pancreatitis, hepatotoxicity and cardiac arrhythmias - making it a relatively unsafe option compared with the other PDCs discussed here. Cyclosporin A and azithromycin can be given to infants 6 months and older. However, in the case of cyclosporin A, an immunosuppressant drug prescribed during transplant procedures, no adequate pediatric studies have been conducted. This PDC was also found to cause hypertension, hyperkalemia, nephrotoxicity, malignancies and hepatotoxicity during clinical trials. In pediatric patients, convulsions have also been observed. Its 33% alcohol content is another factor to be noted before considering administration to infants. The safety of intravenous administration of azithromycin, a macrolide antibiotic used to treat community-acquired pneumonia, has not been established in children below the age of 16 years. However, it is used orally to treat pediatric patients 6 months and older. The most common adverse effects caused by this PDC during clinical trials were mild nausea, vomiting, diarrhea and abdominal pain making it a safe option for pediatric prescription. Loperamide hydrochloride and omeprazole can both only be given to children who are over the age of 2 years. Loperamide hydrochloride, prescribed for symptomatic relief of diarrhea, is contraindicated in pediatric patients who are younger than 2 years because it poses a risk of serious cardiac adverse reactions as well as respiratory depression. Patients between the ages of 2 and 5 should be given the drug in the form of its over-the-counter oral solution, whereas those aged 6 years or older can also be given capsules. This PDC is a good pediatric option because adverse reactions to it during clinical trials were mainly dry mouth, colic, cramps, nausea and constipation. The safety of omeprazole was established in the treatment of pediatric patients above the age of 2 years, for erosive esophagitis and GERD, through pharmacokinetic studies performed in children aged 2–16. Other than fever and respiratory system events, pediatric adverse effects were mild, such as headaches, abdominal pain, nausea, diarrhea and vomiting. Amlodipine besylate is used to treat hypertension and coronary artery disease, whereas clemastine fumarate is an antihistamine used to provide symptomatic relief for allergic rhinitis. Both these PDCs can only be prescribed to children aged 6 years or older, because the safety and efficacy of these drugs in younger patients has not yet been established. During clinical trials, the most common adverse effects of amlodipine besylate were headaches, fatigue, nausea, edema and dizziness, whereas those of clemastine fumarate were sleepiness, dizziness and gastric distress - thus making them two of the safer PDCs discussed here. Given that all of the drugs mentioned in this section are in current use for the treat-

ment of various diseases, they are strong candidates for repurposing as antimalarials. The safer PDCs include esomeprazole magnesium, rocuronium bromide, azithromycin, loperamide hydrochloride, omeprazole, amlodipine besylate and clemastine fumarate. These together need further clinical evaluation.

Use during pregnancy

Pregnant women have an enhanced vulnerability to malaria infection which can lead to considerable risks to the mother, fetus and newborn child. Each FDA-approved drug is categorized based on the safety of its usage during pregnancy. According to the FDA, Category A drugs showed no evidence of risk to the fetus in well-controlled human studies. Category B drugs either showed no evidence of fetal risk in animal studies in the absence of well-controlled human studies, or did not exhibit adverse effects on the fetus in human studies although they might do so in animal studies. Category C drugs lack adequate human studies but posed risks to the fetus in animal studies. Category D drugs exhibited positive evidence of fetal risk in studies of pregnant women. Category C and Category D drugs can still be administered to pregnant women if the potential benefits of using the drug outweigh the potential risks. Category X drugs are those that have also shown positive evidence of fetal risk. However, their benefits do not outweigh the potential risks involved in prescribing them to pregnant women. Therefore, establishing the safety of PDCs during pregnancy is vital in the fight against malaria. For this purpose, we classified all 18 PDCs based on their FDA pregnancy categories. Three PDCs were Category B, twelve were Category C, one was Category D and two were Category X (Fig. 3B). These drug categories are discussed in more detail below.

FDA Category B PDCs

The antibiotic azithromycin, the antiviral ritonavir, and the antihistamine clemastine fumarate are three FDA Category B PDCs (Fig. 3). Studies have shown that exposure to azithromycin during the first trimester did not result in any increase in the risk of cardiac or major congenital malformations in the child [12]. However, a more recent study showed that the use of macrolides, such as azithromycin, early during pregnancy increases the risk of spontaneous abortion [13]. Ritonavir, in combination with lopinavir, has been observed to be well tolerated in pregnancy, and has also been shown to be associated with fewer adverse effects in infants compared with other protease inhibitors [14,15]. Although there are no adequate and well-controlled human studies recognized by the FDA regarding the use of clemastine during pregnancy, first-generation antihistamines have been shown to have no teratogenic effects when used during any trimester of pregnancy [16,17].

FDA Category C PDCs

The 12 FDA Category C PDCs are loperamide hydrochloride, esomeprazole magnesium, omeprazole, cyclosporine A, amlodipine besylate, lopinavir, triamterene, verteporfin, auranofin, gramicidin A, rocuronium bromide and pentamidine isethionate. Literature searches did not provide any relevant information regarding the outcomes of gramicidin A, rocuronium bromide or pentamidine isethionate use during pregnancy. However, extensive information regarding the other drugs is available because of their prior testing in pregnancy studies. A moderate increase in risk of malformations in infants has been associated with loperamide use in early pregnancy [18]. The use of PPIs, such as esomeprazole and omeprazole, during pregnancy has not been reported to be responsible for any increase in risks of spontaneous abortions, congenital malformations or preterm deliveries [19]. Instead, these PPIs brought about reduced spontaneous contractions in the myometrial muscles of pregnant women [20]. Esomeprazole was recently found to also display therapeutic potential to treat pregnancy complications such as pre-eclampsia [21]. Additionally, a study conducted in 2014 concluded that although PPI exposures during pregnancy were originally suspected to increase the risk of hypospadias in male offspring, exposure to this drug class during pregnancy (including omeprazole) did not have any association with the disorder [22]. In pregnant women, Cyclosporin A has been suspected to be associated with premature delivery and low birth weight in infants, although short-term and low dosage uses are still considered safe [23]. The drug has also been reported to be safe to use in the treatment of ulcerative colitis in pregnant women [24]. Although adequate studies of amlodipine use during pregnancy are lacking, a limited study conducted in 2007 concluded that exposure to the drug during the first trimester of pregnancy did not have any teratogenic effects in the fetus [25]. Lopinavir, in combination with ritonavir, was found to have no safety concerns in pregnant women being treated for HIV, even without particular dose adjustments [26]. However, triamterene and other folic acid antagonists used in the treatment of edemas, are suspected to be associated with an increased risk of oral clefts, urinary tract defects, neural-tube defects as well as cardiovascular defects [27]. Verteporfin, used to treat subfoveal choroidal neovascularization, lacks adequate studies conducted in pregnant women. However, the drug was reported to have no adverse effects on the fetus in a few isolated cases [28,29]. Administration of auranofin, a drug used to treat rheumatoid arthritis, to pregnant rabbits led to a reduced intake of food, reduced fetal weights, as well as an increase in the frequency of congenital abnormalities and abortions only at doses ranging between 4.2 to 50 times the recommended human dose.

FDA Categories D and X PDCs

The FDA Category D drug telmisartan is used to treat hypertension and has been reported to interfere with fetal renal function. Thus it is not recommended for use by pregnant women [30,31]. Clomiphene and raloxifene, both FDA Category X drugs, are contraindicated in pregnant women. Clomiphene, used to treat ovulatory dysfunction, has been observed to be associated with inducing luteolysis in guinea pigs and causing pregnancy loss later on [32]. Its use during pregnancy has also been observed to be related to intrauterine growth restrictions [33]. Raloxifene is used to treat osteoporosis and has been shown to cause fetal growth retardation and developmental deviation in pregnant rats. There was an increase in the number of runts, as well as offspring having wavy ribs and kidney cavitations following raloxifene exposure during pregnancy [34].

Potential protein targets of FDA-approved drugs in *P. falciparum*

Although the PDCs considered here all showed antiparasitic activity in the assays performed by Cheng *et al.* and Derbyshire *et al.* [6,7], the drug target mechanisms remain unclear in the context of parasite proteins. Therefore, we assessed the likely primary targets

of the 18 PDCs using the DrugBank database where their protein targets in humans are listed (Table 1). Following this, BLAST searches were performed for each drug–target pair against PlasmoDB to identify orthologs in *Plasmodium*. Sequence alignment of the targets of all PDCs was performed. For those PDCs that had originally been tested against liver-stage *P. berghei*, sequence alignment of targets was also done against *P. berghei*. This enabled us to generate a list of *Plasmodium* proteins that could be equally targeted by the 18 PDCs, a prediction that requires experimental validation as part of drug-repurposing studies.

Thus we short-listed eight PDCs for which the target proteins could be unambiguously identified in *Plasmodium* (Table 1). Of the 15 PDCs tested against blood-stage *P. falciparum*, five (azithromycin, pentamidine isethionate, auranofin, loperamide hydrochloride and amlodipine besylate) had target matches in *P. falciparum* (Table 1). Of the five PDCs tested against liver-stage *P. berghei*, three (cyclosporin A, esomeprazole magnesium, and omeprazole) had target matches in both *P. berghei* and *P. falciparum* (Table 1). The antibiotic azithromycin had four targets, of which three were bacterial proteins whereas the fourth was a human protein (Table 1). The two targets that showed matches in *P. falciparum* were the bacterial proteins 50S rRNA protein L4 and 50S rRNA protein L22, which are important during the early stages of 50S ribosomal assembly. The first target, 50S rRNA protein L4, had two matches in *P. falciparum* (a mitochondrial form and an apicoplastic form of the protein), whereas the second target, 50S rRNA protein L22, had only one match (as an apicoplastic protein) in the parasite (Table 1). This suggests that orthologous proteins for those targeted by azithromycin in bacteria are present in *P. falciparum,* implying that azithromycin has the same targets in both organisms. Protein target information such as this provides greater insights into the potential of these PDCs as antimalarials, and establishes a platform for their experimental validation. Pentamidine isethionate has one target: tRNA [cytosine(38)-C(5)]-methyltransferase, a methyltransferase that acts on cytosine 38 in the anticodon loop of tRNA-Asp. This protein showed a match with the protein DNA (cytosine-5)-methyltransferase in *P. falciparum* (Table 1). Auranofin has two targets, mitochondrial peroxiredoxin-5, involved in intracellular redox signaling, and inhibitor of nuclear factor kappa-B (NF-κB) kinase subunit β, involved in the NF-κB signaling pathway (Table 1). Sequence alignment against the PlasmoDB database showed that in *P. falciparum* the first target matched with the protein 1-cys peroxiredoxin, whereas the second target matched with the protein calcium-dependent protein kinase 2 (CAMK; Table 1). Loperamide hydrochloride has seven targets (Table 1) but only calmodulin (a protein involved in the calcium signal transduction pathway) had a match in *P. falciparum*. Amlodipine besylate has ten targets (Table 1), of which only one showed a match in *P. falciparum*: carbonic anhydrase, a zinc metalloenzyme involved in processes in humans such as respiration, bone resorption and calcification (Table 1). Cyclosporin A has four targets, of which three showed matches in *P. berghei* as well as *P. falciparum*. These were calcineurin subunit B type-2 (a calmodulin-dependent serine/threonine protein phosphatase involved in calcium-dependent signal transduction pathways), peptidyl-prolyl *cis-trans* isomerase A (an isomerase that regulates processes such as transcription, inflammation, apoptosis etc.), and peptidyl-prolyl *cis-trans* isomerase F-mitochondrial (an isomerase involved in mitochondrial metabolism and apoptosis) (Table 1). Esomeprazole magnesium had one target, potassium-transporting ATPase α chain 1 (involved in catalyzing the hydrolysis of ATP coupled with potassium ion exchange across the plasma membrane), the sequence alignment of which, against the PlasmoDB database, showed a match with the protein calcium-transporting ATPase in *P. berghei* and non-SERCA-type Ca^{2+}-transporting P-ATPase in *P. falciparum* (Table 1). Potassium-transporting ATPase α chain 1 is also the first target of omeprazole, and matched with the same proteins in *P. berghei* and *P. falciparum* as mentioned above for esomeprazole magnesium. The second target of omeprazole is an aryl hydrocarbon receptor, with which no matches were observed in either *Plasmodium* parasite. The PlasmoDB IDs of the target matches of all PDCs are provided in Table 1.

Additionally, through literature surveys it was found that other than azithromycin, lopinavir and ritonavir, none of the other PDCs have been tested in humans as antimalarials [35–39]. Clinical testing of azithromycin in combination with chloroquine as a potential antimalarial showed a 28-day PCR-corrected parasitological clearance rate [35,36]. A cure rate of 69.7% was seen on day 28 in clinical trials of azithromycin in combination with dihydroartemisinin [37]. Lopinavir and ritonavir have been tested as a combination drug, and most clinical trials involving them are comparisons with other antiretroviral therapies used to prevent malaria in patients with HIV [38,39]. A study conducted in children with HIV showed that lopinavir/ritonavir-based antiretroviral therapies were associated with a lower risk of recurrent malaria-positive blood smears compared with non-nucleoside reverse transcriptase inhibitor (NNRTI)-based antiretroviral therapies [38]. However, no lowering of confirmed clinical malaria incidence was observed compared with NNRTI-based antiretroviral therapies [38]. The authors concluded that this could result from modification of immune responses to malaria, either through lopinavir/ritonavir-based antiretroviral therapy-caused killing of malaria parasites or via better suppression of HIV [38]. Another study conducted in pregnant women with HIV observed that lopinavir/ritonavir-based treatment did not reduce the risk of placental or maternal malaria compared with treatment based on another antiretroviral efavirenz [39]. The increased response to lopinavir/ritonavir-based antiretroviral therapies in children with HIV was attributed to a difference in age-related antimalarial immunity [38]. Clinical trials focused on lopinavir/ritonavir-based antiretroviral therapy alone are required to fully understand the potential of these drugs as antimalarials.

The proposed PDCs in this study will need to demonstrate satisfactory pharmacokinetics and maintain efficacy. Furthermore, they will need to be tested *in vivo* by measuring their therapeutic efficacy against rodent *Plasmodium* spp. (*P. berghei* and *P. yoelli*), followed by murine models of human-infecting *Plasmodium* spp. (*P. falciparum*), such as NOD-*scid IL2R*γ^{null} or NOD-*scid* β*2microglobulin*^{null} mice engrafted with human erythrocytes [40]. Early clinical development will need to be implemented to test the PDCs by measuring parasite clearance time 36 h after monotherapy [41]. Among the proposed PDCs, nine have been reported to develop resistance in nonmalaria diseases (Table 1). As with all antimalarials, it is therefore likely that resistance against these PDCs will eventually arise. However, given the recommended combination drug therapy mode for malaria, any new

TABLE 1

Target annotation of PDCs in *Plasmodium*[a]

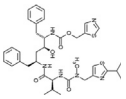
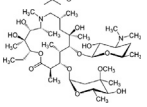
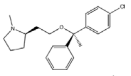
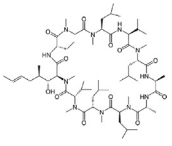
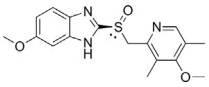
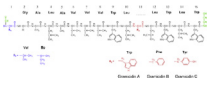
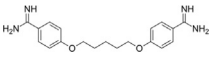
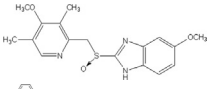
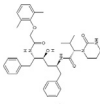
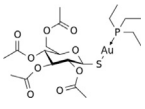
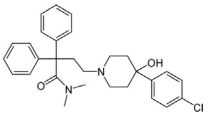
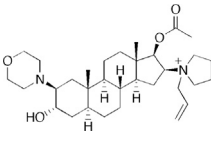
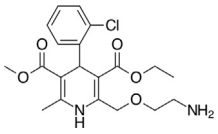
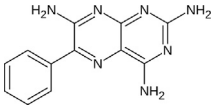
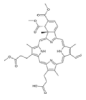
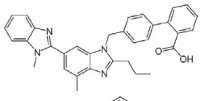
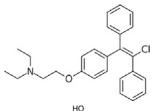
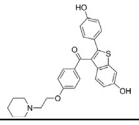
No.	Drug name and range of recommended dosage	Structure	Human Targets	*Plasmodium* Targets	Reported resistance against	Refs
1	Ritonavir (300–600 mg PO q12hr)		1. HIV type 1 protease 2. Nuclear receptor subfamily 1 group I member 2	1. No hits 2. No hits	HIV A17	[44]
2.	Azithromycin (250–600 mg – based on patient's response and disease severity)		1. 23S rRNA 2. 50S rRNA Protein L4 3. 50S rRNA Protein L22 4. Protein-arginine deiminase type-4	1. Absent 2. PF3D7_0822000, PF3D7_API01300 3. PF3D7_1467400 4. No hits	1. *Neisseria gonorrhoeae* 2. Mycobacterium genitalium 3. Campylobacter 4. *Shigella*	[45–48]
3	Clemastine fumarate (1.34–2.38 mg PO q8h)		Human histamine H_1 receptor	No hits	No information	
4	Cyclosporin A (3–6 mg PO q12h)		1. Calcium signal-modulating cyclophilin ligand 2. Calcineurin subunit B type-2 3. Peptidyl-prolyl cis-trans isomerase A 4. Peptidyl-prolyl *cis-trans* isomerase F-mitochondrial	1. No hits 2. PBANKA_1315400, PF3D7_1451700 3. PBANKA_1216500, PF3D7_0322000 4. PBANKA_1216500, PF3D7_0322000	Hepatitis C virus	[49]
5	Esomeprazole magnesium (20 mg orally once daily)		Potassium-transporting ATPase α chain 1	PBANKA_0207000, PF3D7_1211900	No information	
6	Gramicidin A (1–2 drops or ointment 2–4 times daily)		No information	No information	1. *Mycoplasma pulmonis* 2. Surfactin-producing bacteria *(ex: Bacillus subtilis)* 3. Gramicidin S-resistant *Staphylococcus aureus*	[50–52]
7	Pentamidine isethionate (300 mg nebulized once in 4 weeks)		tRNA [cytosine(38)-C(5)]-methyltransferase	PF3D7_0727300	1. *Acinetobacter baumannii* 2. *Saccharomyces cerevisiae*	[53,54]
8.	Omeprazole (20 mg orally once daily)		1. Potassium-transporting ATPase α chain 1 2. Aryl hydrocarbon receptor	1.PBANKA_0207000, PF3D7_1211900 2. No hits	No information	
9	Lopinavir (800 mg daily orally)		Human immunodeficiency virus Type 1 protease	No hits	HIV-A17	[44]
10	Auranofin (6 mg daily orally)		1. Peroxiredoxin-5, mitochondrial 2. Inhibitor of NF-κB kinase subunit β	1. PF3D7_0729200 2. PF3D7_0610600	1. Ovarian cancer cell line 2. Gastric cancer cells 3. Colorectal cancer cells	[55–57]
11	Loperamide hydrochloride (4–16 mg daily orally)		1. μ-opioid receptor 2. δ-opioid receptor 3. κ-type opioid receptor 4. Voltage-dependent P/Q-type calcium channel subunit α1A 5. Pro-opiomelanocortin 6. Calmodulin 7. Nuclear receptor subfamily 1 group I member 3	1. No hits 2. No hits 3. No hits 4. No hits 5. No hits 6. PF3D7_1434200 7. No hits	No information	
12	Rocuronium bromide (0.6–1.2 mg per kg IV)		1. Neuronal acetylcholine receptor subunit α2 2. Muscarinic acetylcholine receptor M2 3. 5-hydroxytryptamine receptor 3A	1. No hits 2. No hits 3. No hits	1. Patients with Schwartz–Jampel syndrome 2. Glucocorticoid concomitant therapy	[58,59]

TABLE 1 (*Continued*)

No.	Drug name and range of recommended dosage	Structure	Human Targets	*Plasmodium* Targets	Reported resistance against	Refs
13	Amlodipine besylate (5–10 mg daily orally)		1. Voltage-dependent L-type calcium channel subunit α1C 2. Voltage-dependent calcium channel subunit α2/δ1 3. Voltage-dependent L-type calcium channel subunit β2 4. Voltage-dependent L-type calcium channel subunit α1D 5. Voltage-dependent L-type calcium channel subunit α1S 6. Voltage-dependent N-type calcium channel subunit α1B 7. Voltage-dependent calcium channel subunit α2/δ3 8. Voltage-dependent L-type calcium channel subunit β1 9. Carbonic anhydrase 1 10. Sphingomyelin phosphodiesterase	1. No hits 2. No hits 3. No hits 4. No hits 5. No hits 6. No hits 7. No hits 8. No hits 9. PF3D7_1140000 10. No hits	No information	
14	Triamterene (100–300 mg daily)		1. Amiloride-sensitive sodium channel subunit γ 2. Amiloride-sensitive sodium channel subunit α 3. Amiloride-sensitive sodium channel subunit β 4. Amiloride-sensitive sodium channel subunit δ	1. No hits 2. No hits 3. No hits 4. No hits	No information	
15	Verteporfin (no information)		No targets	No targets	No information	
16	Telmisartan (80 mg orally daily)		1. Type-1 angiotensin II receptor 2. Peroxisome proliferator-activated receptor γ	1. No hits 2. No hits	No information	
17	Clomiphene citrate (50–100 mg orally once daily for 5 days)		1. Estrogen receptor α 2. Sex hormone-binding globulin	1. No hits 2. No hits	No information	
18	Raloxifene hydrochloride (60 mg orally daily)		1. Estrogen receptor α 2. Estrogen receptor β 3. Serpin B9 4. Trefoil factor 1	1. No hits 2. No hits 3. No hits 4. No hits	Breast cancer cell lines	[60]

[a] Results obtained from BLAST search of the human enzyme target of a given PDC against the PlasmoDB database are listed. All drug structures are from the DrugBank database.

addition to support artemisinin-based drug cocktail will be valuable.

Concluding remarks

The eight PDCs described here that show drug target matches in *Plasmodium* (azithromycin, auranofin, loperamide hydrochloride, amlodipine besylate, cyclosporin A, pentamidine isethionate, esomeprazole magnesium and omeprazole) should be investigated further for their potential as antimalarial candidates. Such explorations could shed light on new parasitic pathways that can be targeted, and will highlight new drug scaffolds that can be repurposed. With several successful examples of drug repurposing [42,43], we hope that similar goals can be achieved in the treatment of malaria with the PDCs detailed in this study.

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