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Drug discovery for malaria: a very challenging and timely endeavor

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The prevalence of resistance to known antimalarial drugs has resulted in the expansion of antimalarial drug discovery efforts. Academic and nonprofit institutions are partnering with the pharmaceutical industry to develop new antimalarial drugs. Several new antimalarial agents are undergoing clinical trials, mainly those resurrected from previous antimalarial drug discovery programs. Novel antimalarials are being advanced through the drug development process, of course, with the anticipated high failure rate typical of drug discovery. Many of these are summarized in this review. Mechanisms for funding antimalarial drug discovery and genomic information to aid drug target selection have never been better. It remains to be seen whether ongoing efforts will be sufficient for reducing malaria burden in the developing world.

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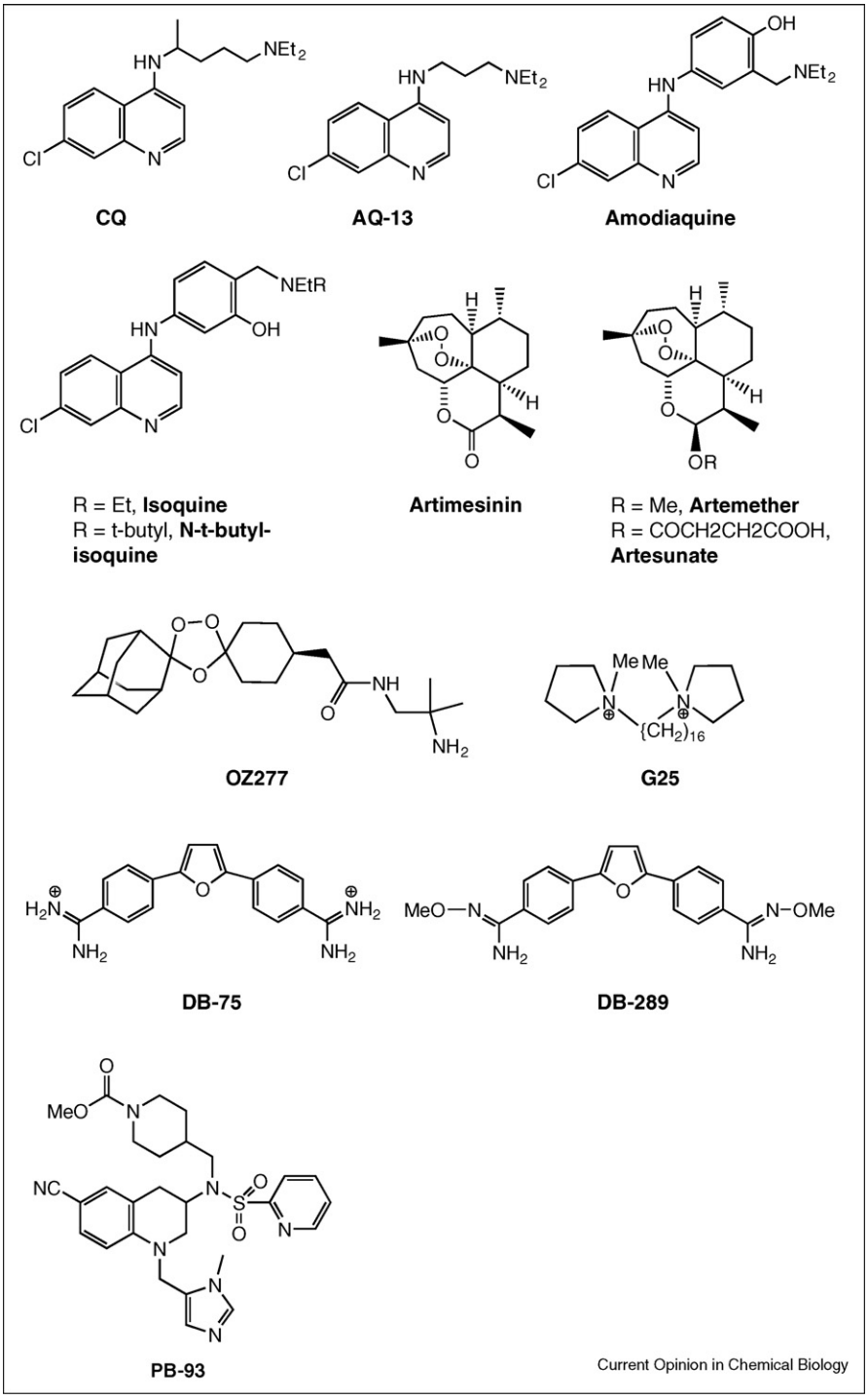
It is common knowledge that malaria is a serious worldwide health problem due to the emergence of parasites that are resistant to well-established antimalaria drugs. Although continued attempts to develop a vaccine for malaria are ongoing, drugs continue to be the only treatment option [1•]. In this review, we will highlight some of the antimalaria drug discovery efforts that are currently being developed at universities, research institutions, and pharmaceutical companies worldwide. Over the past decade, hundreds of reports of antimalarial substances have appeared in the primary and patent literature; however, many of these reports disclose compounds that block the growth of malaria parasites in human erythrocyte cultures at concentrations in the micromolar range. On the basis of the fact that values of ED_{50} (the concentration of compound that causes 50% growth inhibition of cultured malaria parasites) of well-established antimalarial drugs, such as chloroquine and artemisinin, are in the low nanomolar range and emergence of clinically significant resistant parasites occurs when the ED_{50} for the drug increases by ~10-fold, it is probably the case that only compounds that have values of ED_{50} in the low nanomolar range can be considered as significant antimalarial leads. Thus, this review will focus on reports where low nanomolar antimalarial potency has been demonstrated. A second point is that compounds with values of ED_{50} in the micromolar range probably stunt the growth of parasites by binding to multiple targets. In these cases, it will be difficult to take a rational and iterative approach for improving the potency of lead compounds. An example of studies from the author's laboratory is revealing. It has been shown that compounds that are subnanomolar inhibitors of the protein farnesyltransferase enzyme from the malaria parasite are cytotoxic to parasites with values of ED_{50} in the low nanomolar range [2•]. Five structurally distinct low nanomolar inhibitors of human protein farnesyltransferase were found to have no effect on malarial protein farnesyltransferase when tested at concentrations up to 10 μM, yet all of these compounds blocked the growth of parasites in culture with values of ED_{50} in the 2–10 μM range [2•], very likely because of off-target effects.

Drug discovery in general is a very challenging process, but it is especially challenging for antimalarials for several reasons [1•]: (1) it is generally agreed among physicians in malaria endemic countries that drugs for malaria treatment need to be well tolerated and safe in humans, with side effects no worse than some of the best tolerated drugs. This is because of the large number of people who will take antimalarials and the fact that follow-up health care is underdeveloped in places where malaria is prevalent; (2) antimalarials need to be orally bioavailable for ease of administration in a nonhospital setting; (3) because of concern about compliance and the development of resistance, a three-day maximum therapy for cure with once or twice a day dosing is desirable; (4) drugs need to be used in combination to reduce the development of resistance, which increases the number of new drugs that need to be developed; (5) antimalarial drugs need to have a low cost of goods. This last factor is nontrivial since most drugs in use in developed countries are beyond the cost of goods requirement for antimalarials; (6) a good part of antimalarial drug development occurs at research centers that are not ideally structured for drug discovery (i.e. academic and nonprofit research institutions).

Next generation chloroquine analogs

Current thinking is that the 4-aminoquinoline chloroquine (Figure 1) kills malaria by accumulating in the food vacuole of parasites where it interferes with the polymerization of heme into hemazoin. Parasites' resistant to chloroquine have acquired a mutant transporter PfCRT that is capable of reducing intracellular exposure to the drug. Remaining controversies with these ideas are not discussed here. Since resistance is controlled by the binding of chloroquine to one or more parasite macromolecules that are distinct from the parasite-killing target, it seems reasonable that molecules that maintain potency for disrupting heme

Figure 1



Structure of antimalarial agents. See text for further discussion.

polymerization while having reduced affinity for the resistance factors can be found. Krogstad and coworkers have been pursuing AQ-13 (Figure 1), which is a close structural analog of chloroquine. AQ-13, like chloroquine, shows low nanomolar potency against cultures of *Plasmodium falciparum*-infected erythrocytes, even those that have acquired resistance to chloroquine. Recent phase I clinical trials have shown that AQ-13 displays pharmacokinetics and toxicities that are only minimally different than those for chloroquine [3]. Continued searching for chloroquine analogs that work against chloroquine-resistant parasites seems like a very logical plan. Inspired by this line or reasoning, Guy and coworkers have developed robust parallel synthetic methods for the preparation of libraries of 4-aminoquinolines and related molecules [4,5]. Several compounds that display ED_{50} values similar to that of chloroquine were found. These hits need to now pass through pharmacokinetic and toxicology filters to find the most promising candidates.

Amodiaquine (Figure 1) is a 4-aminoquinoline structurally related to chloroquine but effective at killing chloroquine-resistant parasites. Although amodiaquine is an effective antimalarial in patients, its use is compromised by its hepatotoxicity and its ability to cause agranulocytosis [6••]. Current thinking is that amodiaquine is oxidized by one or more cytochrome P450 enzymes to the quinone imine and that the latter is responsible for the toxicity owing to its ability to react with a variety of nucleophiles. Ward and colleagues studied the regioisomer of amodiaquine in which the positions of the groups on the hydroxy aniline ring have been swapped. Isoquine (Figure 1) emerged from this study as a promising antimalarial agent that cannot be oxidized to the quinone imine [6••]. Studies with radioactive compounds showed that much less isoquine is accumulated in the liver of rats than amodiaquine. This suggests that isoquine does not form covalent adducts with liver components. Further optimization led to the discovery of *N-t*-butyl-isoquine (Figure 1) [6••], which is now being transitioned toward antimalarial clinical trials.

Artemisinin and related organic peroxides

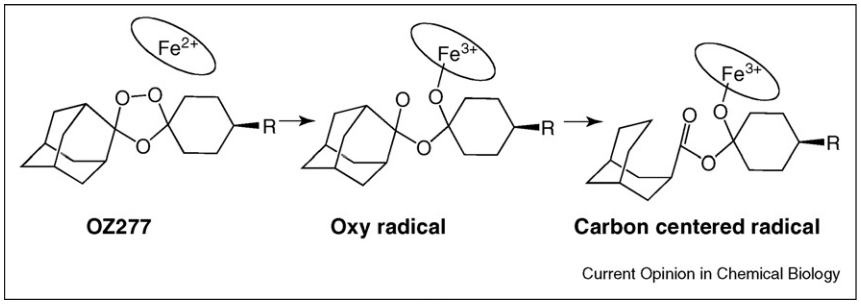
Artemisinin, a plant-derived natural product (Figure 1), is now in wide use for the treatment of malaria. Artemisinin-based combination therapies are now recommended by the World Health Organization for treatment of uncomplicated malaria in regions where resistance to other antimalarials is prevalent. Further optimization of artemisinin-based therapy for malaria is ongoing. A number of semisynthetic routes to prepare artemisinin analogs such as artemether and artesunate (Figure 1) with changes to the δ-lactone portion have been developed with the goal of improving the pharmacokinetic prosperities [7,8]. New combination therapies in which one of the components is an artemisinin-derived antimalarial are either in clinical development or recently approved therapies. These include Pyramax, the artemisinin analog artesunate in combination with the 4-aminoquinoline pyronaridine; Coartem, the artemisinin analog artemether in combination with lumefantrine, an antimalarial drug developed several years ago in China and with an unknown mode of action; DHA-piperaquine, the artemisinin analog dihydroartemisin in combination with the quinoline-based drug piperaquine; the triple drug combination DCA composed of the dihydrofolate reductase inhibitor prodrug chloroproguanil, the sulfa-drug dapsone and the artemisinin analog artesunate (www.mmv.org); ASAQ, the artemisinin analog artesunate in combination with the 4-aminoquinoline amodiaquine (www.dndi.org).

The demand for plant-derived artemisinin may soon exceed the supply. In a remarkable series of bioengineering experiments, Keasling's lab has transplanted plant biosynthetic genes into yeast to allow production of the artemisinin precursor artemisinic acid in yields that appear suitable for large-scale fermentation [9••]. The oxygenation of artemisinic acid to artemisinin will probably have to be carried out by nonfermentation methods since large amounts of the latter are probably toxic to cells. It remains to be seen whether a short and practical conversion of artemisinic acid to artemisinin can be achieved by chemical synthesis.

A second approach to meet the demands for artemisinin-based therapies is to develop a totally synthetic artemisinin analog that can be manufactured at a price competitive with that of the agricultural process. In a remarkable series of studies inspired by the presence of the peroxide function of artemisinin, Vennerstrom *et al.* developed the 1,2,4-trioxolane OZ277 (Figure 1) as a potent antimalarial that has recently transitioned into phase II clinical trials [10••] (www.mmv.org). OZ277 lacks chiral centers and is synthesized in a short and economical fashion by Griesbaum co-ozonolysis involving the joining of *O*-methyl adamantanone oxime with a substituted cyclohexanone in the presence of ozone followed by postozonolysis side chain elaboration [10••].

The mode of parasite killing by artemisinin and related peroxides is heavily debated. At one extreme is the idea that carbon-centered radicals derived from metal-induced scission of the peroxide bond lead to alkylation of a large number of intracellular targets (see Figure 2 for a proposal for OZ277 reactivity). At the other extreme is the proposal that artemisinin acts at a single target, a Ca^{2+}-ATPase known as PfATP6 [11]. Evidence for the former comes from the 'loose' structure–activity relationships observed in the antimalarial activity of artemisinin analogs, that is, activity is maintained even when a large number of diverse structural changes are made to OZ277. Evidence in support of PfATP6 as the major target comes from recent studies showing that parasites harboring a single point mutant in PfATP6 are resistant to artemisinin [12]. Both sets of data seem compelling, so the controversy

Figure 2



Proposed mechanism for heme iron-catalyzed generation of a carbon-centered radical from the antimalarial agent OZ277. The carbon-centered radical is proposed to alkylate a number of parasite proteins. See text for further discussion.

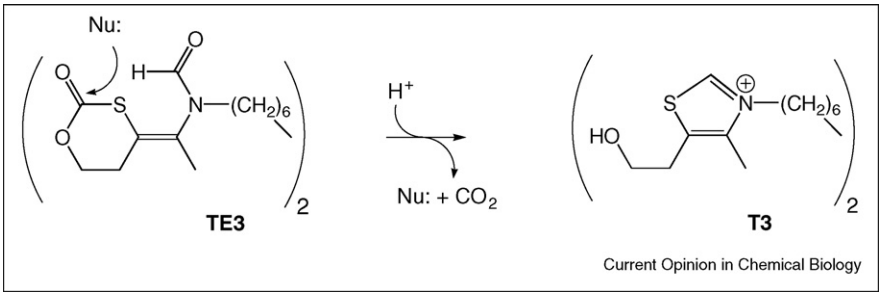
continues. It remains possible that artemisinin and OZ277 kill parasites by different mechanisms.

Novel cationic molecules

It is well established that during rapid parasite growth in infected erythrocytes, phosphatidylcholine biosynthesis is ramped up, presumably as a result of increased membrane biosynthesis by dividing parasites. It appears that choline uptake is required for this phospholipid biosynthesis since choline uptake in infected erythrocytes is much higher than in noninfected cells. Vial and colleagues have been exploring a number of cationic molecules as choline analogs with the ability to block choline uptake. In early studies, the compound G25 (Figure 1) emerged as a bis-cationic molecule with exceptional *in vitro* antimalarial activity (ED_{50} = 0.6 nM) [13]. G25 is ~1000-fold less toxic to mammalian cell lines. Later studies showed that G25 selectively accumulates in malaria-infected erythrocytes [14•]. Treatment of malaria-infected monkeys with low doses of G25 (i.m. 0.15 mg/kg twice daily for eight days) was sufficient to achieve a cure in all five treated animals [14•]. Compounds such as G25 display poor oral bioavailability presumably because they contain permanent positive charges. In a remarkable series of studies, Vial and coworkers reported that the bis-cationic compound T3 (Figure 3) displays potent *in vitro* antimalarial activity with ED_{50}s in the 2–9 nM range [15••]. In the same report they found that the prodrug TE3 (Figure 3) is stable in intestinal fluid, slowly breaks down to T3 in gastric fluid (half-time ~8 hours), and rapidly breaks down in human plasma (half-time ~5 min). The oral bioavailability of TE3 in rats is 16%, and this prodrug is able to cure malaria in monkeys when dosed orally in the 3–27 mg/kg range once a day for four days [15••]. These compounds are exciting leads for antimalarial drug discovery. Whether a malarial choline transporter is the target of these compounds remains to be conclusively established.

Tidwell and coworkers have been studying a family of bis-cationic molecules as antimicrobial agents for several years. One compound, DB-75 (Figure 1), is a broad spectrum antimicrobial that displays low nanomolar potency as an inhibitor of the growth of *Trypanosoma brucei*, the parasite that causes African sleeping sickness. DB-75 is structurally related to pentamidine, a compound known to posses antiprotozoal activity since the 1930s. A major breakthrough came when it was discovered that the diamidoxime prodrug, DB-289 (Figure 1), is orally absorbed and then converted to the active DB-75 in the bloodstream [16]. DB-289 is currently undergoing clinical trials for the treatment of African sleeping sickness. In the meantime, DB-289 was found to display good potency against malaria [17••]. Given the fact that DB-289 had already gone through phase I clinical trials for the

Figure 3



Generation of the active antimalarial agent T3 from the prodrug TE3. See text for further discussion.

treatment of African sleeping sickness, a clinical trial to test this compound for efficacy in malaria was initiated in Thailand [17••]. The study showed that DB-289 exhibited good efficacy in clearing patients of *P. falciparum* and *P. vivax* infections. However, because efficacy required ~7 days, it was decided to halt clinical testing of DB-289 for malaria with the hope that a more active analog can be found that can achieve a cure of malaria in ~3 days (in order to meet the antimalarial drug target parameters listed above).

The mode of microbial killing by bis-amidines like pentamidine and DB-75 is unknown. It is well established that these compounds accumulate to high concentrations in *Trypanosoma brucei* [18] and probably in *P. falciparum*-infected human red blood cells. Given the high intracellular concentration, multiple targets seem likely.

Piggyback medicinal chemistry

The classical approach for the development of antimalarials is to focus on parasite targets for which no human host homolog exists, thereby minimizing the chance of drug toxicity. Although this concept certainly is rational, the downside is that the process of drug development starting only from a parasite-specific target is a very long and expensive process. The piggyback approach is to explore classes of drug molecules as antimalarials that have already been thoroughly evaluated as drug leads for diseases that have been addressed by major pharma. One example is protein farnesyltransferase inhibitors that have been extensively developed over the past 15 years as anticancer agents. It was shown several years ago that *P. falciparum* contains protein farnesyltransferase and that inhibitors of this enzyme are significantly more toxic to parasites than to human cells. Extensive clinical trials of protein farnesyltransferase inhibitors have shown that they are well tolerated in man during two to three weeks of continuous dosing, so it seems that an inhibitor that is selective for the malaria enzyme is not needed, especially since malaria treatment is typically a few day course of drug administration. After testing all of the major classes of protein farnesyltransferase inhibitors that have been developed at major drug companies, Gelb, Van Voorhis, and their colleagues found that the tetrahydroquinoline class of compounds, developed at Bristol Myers Squibb, display low nanomolar potency for killing cultured *P. falciparum* [2•] (PB-93 in Figure 1 is one example). The mode of parasite killing is very likely due to inhibition of protein farnesylation since parasites that have become resistant to tetrahydroquinolines contain a mutant protein farnesyltransferase that displays a ~10-fold reduction in affinity for these inhibitors [19]. The current status of protein farnesyltransferase inhibitors as antimalarials is that they can cure malaria-infected rodents [2•], but further work is needed to reduce the metabolic instability of this class of drug leads.

Miscellaneous drug discovery efforts

A number of promising antimalarial drug discovery projects are being funded by the Medicines for Malaria Venture (MMV), a nonprofit organization focused on antimalarial drug discovery. These projects are only listed here since results have not been published. These include the following: (1) inhibitors of cysteine proteases involved in the degradation of hemoglobin (falcipain inhibitors); (2) inhibitors of dihydrofolate reductase; (3) Inhibitors of the enoyl-acyl carrier protein component of type II fatty acid synthase. Additional information about these projects can be found at http://mmv.org.

Closing remarks

There is considerable progress in the development of new antimalarials owing in large part to the availability of new funding mechanisms for drug discovery research (i.e. The Bill and Melinda Gates Foundation, The Wellcome Trust, Irish Aid, USAID, and others). What seems to be working more than anything else in the past decade is the development of Public–Private Partnerships (PPPs) in which Pharma contributes drug discovery skills, whereas academic and nonprofit institutions contribute basic biology leading to drug target selection and field expertise [20•]. Many of the most successful antimalaria drug development programs are proceeding through the Medicines for Malaria Venture [21•], which started in 1999. It is fair to say that most of the promising new antimalarial agents in clinical trials are either resurrected agents from the past antimalarial programs that were halted years ago, often in Pharma because of budget restrictions, or structural analogs of known antimalarials (i.e. chloroquine and artemisinin analogs). The failure rate for novel antimalarials is high and will probably remain high in the foreseeable future. There is valid concern that the number of programs focusing on novel antimalarials is insufficient to sustain the antimalarial drug pipeline. Having said all of that, the antimalarial drug discovery funding mechanisms and availability of information for drug target selection have never been better.

Acknowledgements

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