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Review Article

NOVEL ANTIMALARIAL AGENTS AND TARGETS: AN OPTIMISM OVER RESISTANCE

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ABSTRACT

Malaria is a complex disease with ample of host–parasite interactions. Due to long terminal elimination half-life, a shallow concentration-effect relationship, and mutations a drug becomes vulnerable to resistance development. Identifying and emphasizing new targets against malaria is surpassingly desired. These varied new targets will provide important new drugs in the future. *P. falciparum* 1–deoxy–D–xylulose–5–phosphate reductoisomerase plays a role in isoprenoid biosynthesis in the malaria parasite, making this parasite enzyme an attractive target for antimalarial drug design. Erythrocytic schizogony is targeted by artemisinins. Interfering with the schizont stage and release of merozoites are potentially promising but unexploited strategies. Many compounds target a host cell enzyme rather than a molecule encoded by the parasite. As the objective of malaria treatment moves from control to elimination, targeting sexual development and sporogony are being extensively worked. Drugs that block the infectivity of the mature sexual form of the gametocyte will be particularly important. Protozoan aquaporins are increasingly recognized as potential drug targets for antiprotozoan drugs. It is hypothesized that *Plasmodium* aquaglyceroporin provides the pathway for glycerol uptake into the malaria parasite. Parasitic AQP is considered to be an attractive target for drug treatment since it has sequence differences compared to that of human AQP. As per WHO Malaria Report 2013, highly cost-effective strategies for antimalarial research are recommended. For the discovery of novel classes of drugs focus on cellular function as a system should be made rather than on the level of the single process or molecule.

Keywords: Antimalarials, *Plasmodium falciparum,* resistance, targets.

INTRODUCTION

Malaria, a parasitic infection transmitted by mosquitoes, has afflicted humans over the millennia. Over 40% of world's population live in malaria endemic areas.[1] Malaria, being a disease of the distant past, has proved to be an alarming restriction to the cultural and socioeconomic progress of man in the tropical, sub-tropical and monsoon prone zones of the world. It is one of the major public health problems in the developing countries. Recent estimates indicate that about 300-500 million clinical cases and about 1.5-2.7 million deaths occur world- wide annually due to it. During 2006, India contributed 83% of total malaria cases in SE Region. Malaria is prevalent in all parts of country especially areas below 5000 feet from sea level. Some of the states contribute about 90% of the total malaria in the country. These states include Madhya Pradesh, Orrisa, Andhra Pradesh, N. E. States, Bihar, etc.[2]

An estimated 207 million cases (uncertainty interval, 135–287 million) and 627 000 malaria deaths (uncertainty interval, 473 000–789 000) are estimated to have occurred in 2012. An estimated 3.4 billion people were at risk of malaria in 2012. Of this total, 2.2 billion were at

low risk (<1 reported case per 1000 population), of whom 94% were living in geographic regions other than the African Region. The 1.2 billion at high risk (>1 case per 1000 population) were living mostly in the African Region (47%) and the South-East Asia Region (37%). If India is excluded from global totals, then domestic government malaria spending rose at a rate of 3% per year between 2005 and 2012.[3]

For the last 50 years, there have been two main categories of antimalarial agents employed in treatment of malaria. These include antifolates and cinchona alkaloids or the quinoline-containing drugs. They are targeted at the asexual erythrocytic phase of the parasite. Apart from Artemisinin derivatives, resistance to all antimalarials has been recorded.[4,5] Chloroquine has lost efficacy against *P. falciparum* because of development of drug resistance for dihydrofolate reductase inhibitors. Proguanil and Pyrimethamine resistance may be induced from a single large dose.[6]

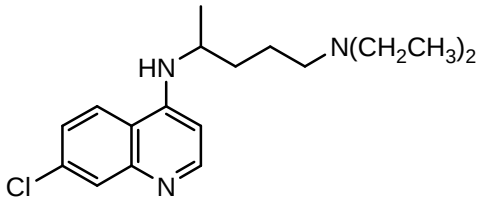
Resistance to Malaria

Resistance to antimalarial drugs is proving to be a challenging problem in malaria control in most parts of the world. Since early 60s the sensitivity of the parasites to chloroquine, the best and most widely used drug for treating malaria, but due to resistance it has been on the decline. Newer antimalarials were discovered in an effort to tackle this problem, but all these drugs are either expensive or have undesirable side effects. Moreover after a variable length of time, the parasites, especially the *falciparum* species, have started showing resistance to these drugs also.

Resistance is defined as "Drug resistance is the ability of the parasite species to survive and/or multiply despite the administration and absorption of a drug given in doses equal to or higher than those usually recommended but within the limit of tolerance." The due to long terminal elimination half-life, a shallow concentration-effect relationship, and mutations a drug became more vulnerable to develop resistance. Drug resistance is most commonly seen in *P. falciparum*. Only sporadic cases of resistance have been reported in vivax malaria. Resistance to chloroquine is most prevalent, while resistance to most other antimalarials has also been reported.[7]

Resistance to chloroquine revolutionalised the treatment of malaria. Resistance began from 2 epicentres – Columbia (South America) and Thailand (South East Asia) in early part of 1960s. Since then, resistance has been spreading worldwide and reached the Indian state of Assam in 1973. Resistance is convened by a stable mutation which is transferred to the progeny. It involves multiple mutations which mean that resistance may be partial also.

Chloroquine (1) acts by getting accumulated in the food vacuole where it inhibits heme polymerase. Resistant strains act by effluxing the drug by an active pump mechanism and release the drug at least 40 times faster than sensitive strains, thereby rendering the drug ineffective. There is also an increase in the surface area of the resistant parasites, permitting more efficient pinocytosis. Binding of chloroquine with haemoglobin breakdown product to form toxic complexes is also prevented. This esistance is maintained throughout the whole life cycle and is transferred to the progeny. Cross-resistance has been demonstrated with other 4-amino quinolines and mepacrine, but not to quinine, mefloquine, PABA blockers or antifolates.[8]



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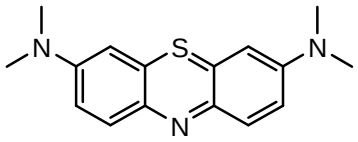
Many approaches to antimalarial drug discovery are available. These approaches must take into account specific concerns, in particular the requirement for very inexpensive and simple to use new therapies and the need to limit the cost of drug discovery. The consideration of new chemotherapeutic targets in malaria research technologies and genomics is most likely to identify new classes of drugs. A number of new antimalarial therapies will likely be needed over the coming years, so it is important to pursue multiple strategies for drug discovery.[9]

Novel Leads As Antimalrialas

8-Aminoquinolines

The discovery of the chemotherapeutic effects of methylene blue (2) leads to the development of synthetic quinoline-based antimalarial drugs on human malaria by Guttman and Ehrlich. It was found that replacing the methyl group with a basic side chain improved activity.[10] Tafenoquine (etaquine, WR 238605) (3) was then introduced for development. Another 8-aminoquinoline in

preclinical trials is CDRI 80/53 (4). This derivative differs from primaquine only by the 2,4-dihydrofuran group present in the basic side chain anchored onto the quinoline nucleus in the 8-position.[11]



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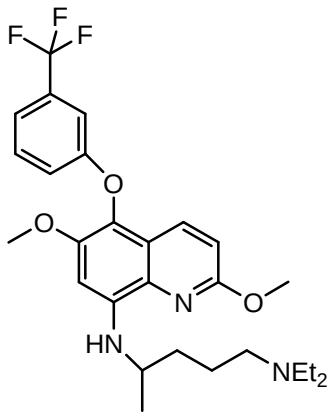

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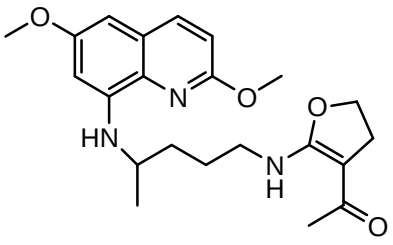

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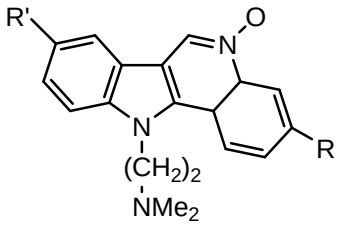
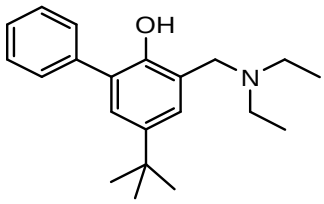
4-Aminoquinolines

Attempts to restrict the spatial flexibility of the 4-aminoquinolines resulted in the synthesis of indoloquinoline (5). Structure–activity studies with these indoloquinolines revealed that the basic side chain and the ring N-oxide are critical for activity (5a). Substitutions at positions 7–10 were not essential. The potent analog in these studies was the 8-nitro compound (5b).[12]



5a R=Br, R'=MeO
5b R=Cl, R'=NO_2

Modification of the α-(dialkyl amino)-*o*-cresol structure (6) led to (7), which was more potent than chloroquine or amodiaquine in both the treatment and prophlyaxis of malaria. Werbel et al. examined a series of hybrid structures of (7) compared to amodiaquine by incorporation of the 7-chloroquinoline moiety expecting that would enhance activity further. A quantitative structure–activity relationship (QSAR) study was used to establish the most suitable substituents on the phenyl ring. The *p*-chlorophenyl group conferred maximal potency with tebuquine (8). Tebuquine was found more potent than chloroquine.



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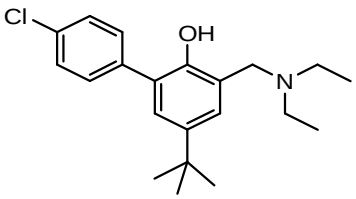

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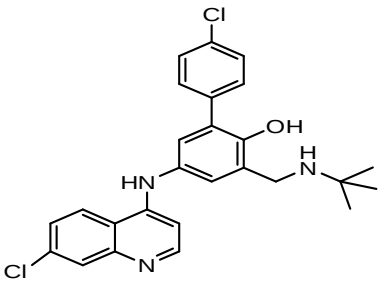

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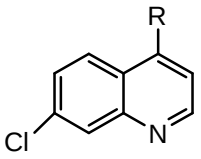
Short-Chain Chloroquine Analogs

Krogstad et al. had synthesized a series of 4-aminoquinoline chloroquine analogs with a range of substituents at the 7-position of the quinoline ring and variable length diaminoalkyl side chains. Data on antimalarial activity suggested that compounds with diaminoalkyl side chains shorter than four carbons or longer than seven carbons were active against chloroquine-susceptible, chloroquine-resistant, and multiresistant strains of *P. falciparum* in vitro and exhibited no cross-resistance with chloroquine. Four of these compounds, possessing a diethylaminoethyl, diethylaminopropyl, dimethylaminoisopropyl and diethylaminoisopropyl side-chains (9a–d) were selected for further studies. All four of these compounds showed significantly lower IC_{50}'s against the chloroquine-resistant K1 strain than was observed for chloroquine.[13]



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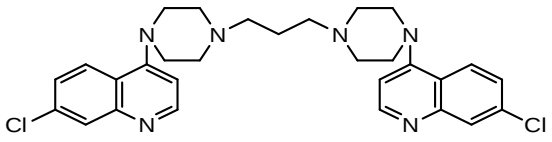
9a R= $NHCH_2CH_2CH_2N(C_2H_5)_2$
9b R= $NHCH_2CH_2N(C_2H_5)_2$
9c R= $NHCH(CH_3)CH_2N(CH_3)_2$
9d R= $NHCH(CH_3)CH_2N(C_2H_5)_2$

Bisquinolines

Bisquinolines are compounds that contain two quinoline nuclei combined through an aliphatic or aromatic linker. Such agents include bis(quinolyl)piperazines, such as piperaquine (10), dichloroquinazine (11) (12,278RP), and 1,4-*bis*(7- chloro-4-quinolylamino)piperazine) (12).[14]



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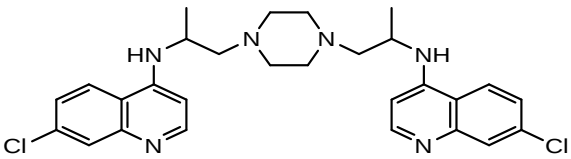

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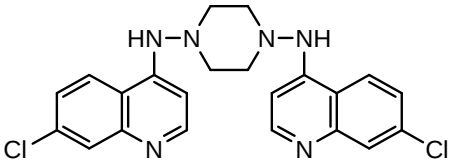

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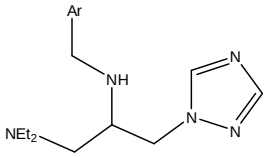
In 2007, nitrogen-analogues of glycerol, ß-blockers, were introduced as a novel class of antimalarials.[15] Prior to this, the well-known ß-blocker propranolol was shown to inhibit infection of erythrocytes by *P. falciparum*, as well as to reduce the parasitaemia of *P. berghei* infections in vivo.[16,17] Due to the biological potential of these compounds, continuous efforts have been devoted to the preparation of structurally diverse analogues bearing a functionalized propane skeleton. More recently, 1,2,3-triaminopropanes (13), 2-amino-3-arylpropan-1-ols (14) and 1-(2,3-diaminopropyl)-1,2,3-triazoles (15) have been reported as a new class of antimalarial compounds.[18,19]



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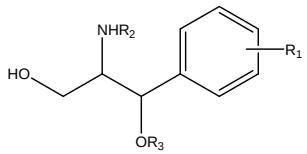

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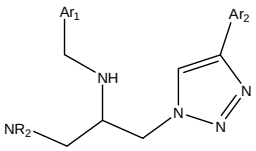

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NEWER TARGETS

Reductoisomerase

P. falciparum 1–deoxy–D–xylulose–5–phosphate reductoisomerase (PfDXR) plays a role in isoprenoid biosynthesis in the malaria parasite and is absent in the human host, making this parasite enzyme an attractive target for antimalarial drug design. From the parallel in silico and in vitro drug screening, it was evident that only a single compound demonstrated reasonable potential binding to DXR, inhibited DXR in vitro and inhibited *P. falciparum* growth,

without being toxic to human cells. Its potential as a lead compound in antimalarial drug development is therefore feasible. The newer analogues of known antimalarial natural products can be screened against malaria, which may then lead towards the rational design of novel compounds that are effective against a specific antimalarial drug target enzyme, such as PfDXR. The rational design of novel compounds against a specific antimalarial drug target enzyme can be untaken by adopting a coupled in silico and in vitro approach to drug discovery.[20,21]

There is a clear need for the identification and development of new classes of anti-malarials, i.e. belonging to novel chemical series and with novel modes of action. The current reality is that much drug discovery is still focused on a limited number of historic targets (primarily the folate and haemoglobin degradation pathways), and primarily on the blood stage of the parasite's life cycle. There is a wealth of untapped targets in the various developmental stage proteomes that might be targeted. As the effort against malaria moves from the control phase to elimination, parts of the parasite life cycle beyond the blood stages will need to be targeted.[22]

Targeting Erythrocytic Schizogony

In the control phase, the focus is on treating asexual blood stages. A key requirement in most target product profiles is rapid parasite kill. The action of the artemisinins is attributed to their activity throughout the erythrocytic stage. Activated artemisinins form adducts with a variety of biological macromolecules, including haem, translationally controlled tumour protein (TCTP) and other higher-molecular-weight proteins.[23] Here we show that artemisinins, but not quinine or chloroquine, inhibit the SERCA orthologue (PfATP6) of *Plasmodium falciparum* in Xenopus oocytes with similar potency to thapsigargin (another sesquiterpene lactone and highly specific SERCA inhibitor).[24] Similarly, interfering with the schizont stage and interrupting the release of merozoites are potentially promising but unexploited strategies. Other targets at this stage may include ring stage particularly focussing on merozoite invasion, trophozoite stage and schizonts.

Host cell targets

The role for host erythrocyte enzymes in parasite development is recently been studied.[25,26] Many compounds may target a host cell enzyme rather than a molecule encoded by the parasite. Targeting host cell (erythrocyte or hepatocyte) enzymes that are required for parasite survival is an additional, as yet unexploited, therapeutic strategy. Recent research indicates that such potential host cell targets include enzymes that are well-established targets in other pathologies (e g, protein kinases in cancer). Some basic research must be done to endorse this approach.

Liver Stage Target

The priority should be given to understand the biology of liver stage parasites, especially the hypnozoites that act as a reservoir for *P. vivax*. Drug activity against hypnozoites will become crucial during elimination. The exact definition of a hypnozoite is not clear. It is sugessted to develop simple markers to identify hypnozoites from active, infected hepatocytes (preferably in human liver cells). This would be a key step to developing screens against the dormant stages. Rudimentary assays already exist for liver stages that do not form hypnozoites: *P. falciparum*, *Plasmodium yoelii*, and *Plasmodium berghei*.[27] Only primary hepatocytes appeared suitable for *P. falciparum*. They have very low permissivity for infection.[28] Stable and infectable hepatocyte lines and standardized conditions for culturing hepatocytes need to be developed, because primary human liver cells are of variable quality and lose their differentiation in culture. The recent study by Bathia and colleagues that use cryopreserved hepatocytes is of potential value for all liver stage models.[29]

Targeting sexual development and sporogony

As the objective of malaria treatment moves from control to elimination, the types of drugs required are changing. Drugs that block the infectivity of the mature sexual form of the gametocyte will be particularly important. Gametocytes are emerging as a novel target to interrupt transmission of the parasite. This may be either the sole activity or an additional activity in the compound.[30] There will be some ethical and practical issues: a purely anti-gametocyte drug would not directly benefit the patient and so would impose a high safety requirement on the compound. Recent research has identified crucial roles for several enzymes in sexual development. Most possible target processes to attack gametocytes include Gametocytogenesis, Egress and subsequent gametogenesis mechanisms. In addition, new assays for gametocytocidal activity have been developed and are presently used for large compounds screening.[31,32]

Aquaporins

Aquaporins, members of membrane proteins discovered and characterized within the past 20 years, are the mechanism through which water is transported through living membranes. The presence of aquaporins explains disease etiology related to water physiology and presents new pharmacogenomic targets.[33]

Protozoan AQPs are increasingly recognized as potential drug targets or transport routes for antiprotozoan drugs.[34,35,36] Physiological roles suggested for protozoan AQPs include metabolism, volume regulation, osmotic stress and osmotaxis.[34,37-42] The PbAQP plays an important role in the blood-stage development of the rodent malaria parasite during infection. It is hypothesized that *Plasmodium* aquaglyceroporin provides the pathway for glycerol uptake into the malaria parasite. The PbAQP provides the pathway for the entry of glycerol into *P. berghei* and contributes to the growth of the parasite during the asexual intraerythrocytic stages of infection.[37] PfAQP is considered to be an attractive target for drug treatment since it has considerable sequence differences compared with that of human AQP. In addition, the lack of functional variability suggests a constant protein core, which may restrict parasite populations from evading therapeutic pressure of potential PfAQP inhibitors.[38] The recently discovered AQPs in parasitic protozoa open a new paradigm for water research in these pathogenic organisms and points to its use as a potential drug target. Despite the obstacles in drug development, namely the lack of crystallographic information and the absence of 3D structures, potential drug ligands and inhibitors will be developed using biophysical and computational techniques and proteomic tools.

Serine Proteases

Malarial proteases are a group of molecules that serve as potential drug targets because of their essentiality for parasite life cycle stages and feasibility of designing specific inhibitors against them. Proteases belonging to various mechanistic classes are found in P. falciparum, of which serine proteases are of particular interest due to their involvement in parasite-specific processes of egress and invasion. In *P. falciparum*, a number of serine proteases belonging to chymotrypsin, subtilisin and rhomboid clans are found.[43]

CONCLUSION

As per WHO malaria report 2013, it is currently recommended to develop highly cost-effective strategies for the use of antimalarial medicines for the prevention of morbidity, targeting groups at high risk of *Plasmodium falciparum* malaria, in areas of moderate to high malaria transmission. We conclude that focusing on cellular function as a system rather than on the level of the single process or molecule will facilitate the discovery of novel classes of drugs. The ambitious target of malaria elimination will only be achieved with the development of new drugs carefully targeted at the key challenges that exist for both control and elimination.

The increasing awareness of the problems posed by multidrug-resistant malaria, and of the need to understand the basis of resistance and develop new chemotherapeutic strategies and drugs, has spurred a rapid increase in the volume of research being applied to this area.

The topics cited previously illustrate the tremendous gains that can be achieved through the application of genetic, genomic, proteomic, biochemical, synthetic or medicinal chemistry, or structural biology approaches.

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