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Antimalarial Drugs – A Review

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ABSTRACT

Malaria remains one of the most important diseases of man with over half of the world's population at a risk of infection and 1-2 million deaths annually. The emergence and rapid spread of of Chloroquine resistant strains of Plasmodium falciparum has dramatically reduced the Chemotherapeutic options. It is important to recognize that antiparasitic drug discovery differs in many ways from drug discovery for the chronic conditions that have become a primary focus of the pharmaceutical industry today. A short review of the antimalarial drugs currently used in human clinics reported. Amodiaquine (AQ) (2) is a 4-Aminoquinoline antimalarial that can cause adverse effects including agranulocytosis and liver damage. This study describes the synthesis of new 4-Aminoquinoline derivatives and evaluation of their activity against Chloroquine-sensitive strain of Plasmodium falciparum invitro and Chloroquine-resistant N-67 strain of Plasmodium yoelli invivo. It is clear that Chloroquine resistant parasites accumulate fewer drugs than sensitive strains. It has been synthesized a new series of Chloroquine analogues where the diethylamino isopentyl function of the side chain has been replaced by shorter side chains, containing metabolically more resilient terminal secondary and tertiary alkyl amino group.

KEYWORDS: Malaria, Chloroquine, chemotherapeutic, antiparasitic drug discovery.

INTRODUCTION

Malaria remains one of the most important diseases of man with over half of the world's population at risk of infection. It remains a tremendous health burden in tropical areas causing 24.3 billion episodes of clinical illness and 0.86 million deaths in 2009, with annual death rates up to 93% of affected severe malaria.1But malaria is by no means a problem for developed countries, where it was defeated long time ago. Despite the importance of this disease, investment into the treatment and control of malaria is modest. As a consequence, the number of deaths due to malaria is gradually increasing as available antimalarial drugs become less effective due to advance of multi-drug resistance. Although much of the current efforts are directed towards the identification of novel chemotherapeutic targets, we still don't fully understand the mode of action and the mechanism of resistance to the Quinoline compounds, knowledge that would greatly assist the design of novel, potent and inexpensive Quinoline antimalarials. Chloroquine (CQ) and other Quinoline antimalarials have been mainstays of malaria chemotherapy for much of the past 40 years. The success of these drugs was based on excellent clinical efficacy, limited host toxicity, ease of use and simple, cost effective synthesis. However, these drugs have been seriously eroded in recent years mainly as a result of the development and spread of parasite resistance to CQ and related compounds.2

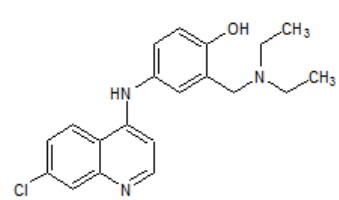
The World Health Organization (WHO) estimates that malaria costs some 1.3% of the annual gross domestic product of the states in which the disease flourishes; their economies have been cut by more than 30%. Today, malaria is transmitted in more than 100 countries. Some 500 million people in Africa, India, South-East and South-America are infected with malaria, with an estimated 2.5 million deaths annually, and about a million of these being children under the age of five.3In 1897, Ronald Ross reported that the parasite Plasmodium could infect a female mosquito, thus showing the complete parasitic cycle. Of these four species, Plamodium falciparum is responsible for the most severe form of malaria, Cerebral malaria, and cause the majority of morbidity case.4Chloroquine (CQ) has been the drug of choice for antimalarial chemotherapy since its discovery as it was safe, affordable and effective but the emergence and rapid spread of P. falciparum and other related antimalarials has dramatically reduced the therapeutic options. CQ resistance in P. falciparum is associated with mutations in the digestive vacuole transmembrane protein PfCRT5.

Life Cycle of Malarial parasite

A female Anopheles mosquito carrying malaria-causing parasites feeds on a human and injects the parasites in the form of sporozoites into the bloodstream. The sporozoites travel to the liver and invade liver cells. Over 5-16 days, the sporozoites grow and give thousands of haploid forms, called merozoites, per liver cell. The merozoites exit the liver cells and re-enter the bloodstream, beginning a cycle of invasion of red blood cells, asexual replication, and release of newly formed merozoites from the red blood cells repeatedly over 1-3 days. This multiplication can result in thousands of parasite-infected cells in the host bloodstream, leading to illness and complications. Some of the merozoite-infected blood cells leave the cycle of asexual multiplication. Instead of replicating, the merozoites in these cells develop into sexual forms of the parasite, called male and female gametocytes, that circulate in the bloodstream. When a mosquito bites an infected human, it ingests the gametocytes. In the mosquito gut, the infected human blood cells burst, releasing the gametocytes, which develop further into mature sex cells called gametes. Male and female gametes fuse to form diploid zygotes, which develop into actively moving ookinetes that burrow into the mosquito midgut wall and form oocysts. Growth and division of each oocyst produces thousands of active haploid forms called sporozoites. After 8-15 days, the oocyst bursts, releasing sporozoites into the body cavity of the mosquito, from which they travel to and invade the mosquito salivary glands. The cycle of human infection re-starts when the mosquito takes a blood meal, injecting the sporozoites from its salivary glands into the human bloodstream.

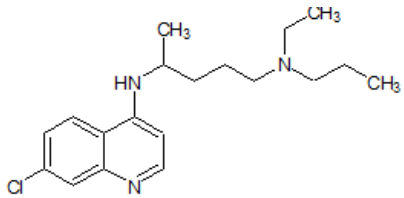
A Brief Review

Amodiaquine (AQ) is a 4-aminoquinoline antimalarial which is effective against many Chloroquine-resistant strains of P. falciparum. However, clinical use of AQ has been severely restricted because of associations with hepatotoxicity and agranulocytosis.6,7 Paracetamol (4-hydroxyacetanilide) contains a p-Hydroxyanilino moiety, which is believed to undergo P-450 catalyzed oxidation to a chemically reactive Quinoneimine. Amodiaquine also contains this functionality and might be expected to undergo enzymic oxidation to a reactive metabolite, Amodiaquine Quinoneimine (AQQI) or Semi-Quinoneimne (AQSQI) with subsequent conjugate addition of glutathione8 which on subsequent binding to cellular macromolecules could affect cell function directly or immunological mechanism.

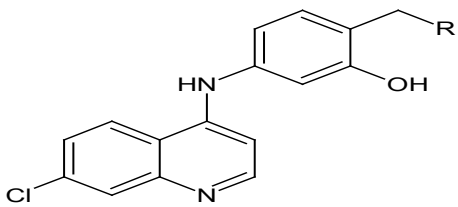


Choroquine (1)

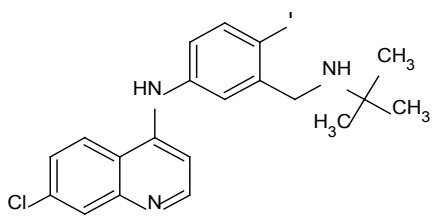
Amodiaquine (2)
Isoquine (3), R = NEt2 (4) R = NHEtBu , Desthyl Isoquine (5) R = NHEt



Amodiaquine (2)

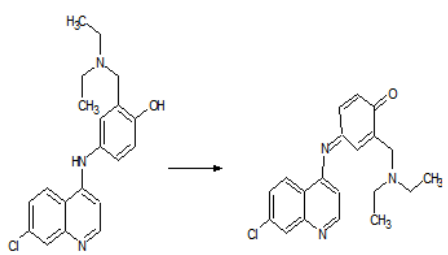


Isoquine (3), R = NEt2 (4) R = NHEtBu , Desthyl Isoquine (5) R = NHEt



4'-Fluoro,N-tertbutyl analogue (6) [FAQ- 4,(6)]

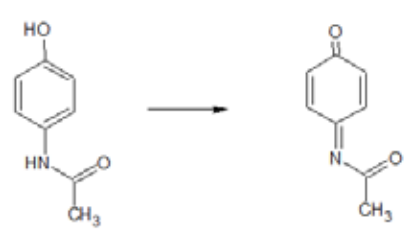
In the case of Paracetamol, it has been shown that introduction of fluorine into the aromatic nucleus increases the oxidation potential of the molecule and thereby blocking the in vivo oxidation of the molecule to cytotoxic Quinoneimine.9 Bioactivation of Amodiaquine and Paracetamol to toxic Quinoneimine by P-450



Amodiaquine (AQ) Amodiaquine Quinoneimine (AQQI)

Binding to Cellular Proteins Hepatotoxicity and Agranulocytosis

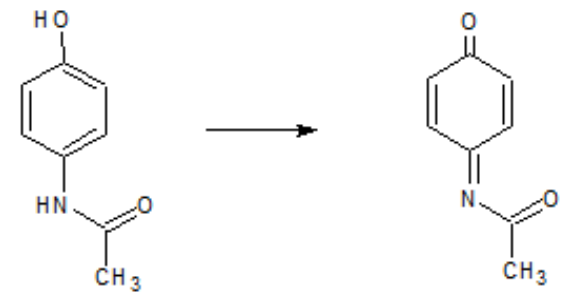
Binding to cellular Macromolecules Hepatotoxicity by P-450



N-Acetyl-p-benzoquinoneimine (NAPQI

Similar to Paracetamol, the incorporation of fluorine atoms into the 4-Hydroxyanilino side chain of Amodiaquine produces compounds with greater oxidative and metabolic stability From the previous work, it was noted that in Amodiaquine and Tebuquine series of 4-Aminoquinoline analogues, the presence of 4'-Hydroxyl group within aromatic ring imparts greater inherent antimalarial activity against Chloroquine resistant parasites than the corresponding deoxo analogues. Interchange of the hydroxyl group and the Mannich side chain provides a means of preventing oxidation to toxic metabolites while retaining possible important bonding interactions with the aromatic hydroxyl function. In this, it has been described the synthesis, antimalarial activity and metabolism of the prototype Isoquine (3 ISQ 1), an Amodiaquine regioisomer that cannot form toxic metabolites by simple oxidation and which is potent against Chloroquine resistant parasites in vitro (Scheme 2).

Binding to cellular macromolecules hepatotoxicity. Scheme 2: Redesign of Amodiaquine



Amodiaquine Isoquine [ISQ 1]

Studies on the mechanism underlying the drug resistance is consequence of decreased accumulation of the drug as a result of enhanced efflux, reduced uptake or a combination of both.10 Accumulation of hematin is detrimental to the parasite survival. Recent reports have shown that close analogues of CQ and its derivatives are active against CQ-resistant parasite strains,11 strongly suggesting that the resistance mechanism does not involve any change to the target of this class of drug but involves a compound specific resistance. Based on this premise, a number of groups have developed short-chain analogues of Quinoline derivatives, which are significantly more potent than CQ against a CQ-resistant strain of P. falciparum in invitro studies.12

The combination Therapy is identified as a strategic and viable option and offers two important potential advantages. First, the combination should improve antimalarial efficacy, providing additive or ideally, synergistic antiparasitic activity. Second, the use of combination therapy should slow the progression of parasite resistance to the new agents. This latter factor is a key consideration as we attempt to develop new therapies that will retain activity for a long period (e.g. combination of Sulfadoxine/Pyremethamine with Amodiaquine,

Chloroquine- A Drug of Choice

Chloroquine (CQ) [7-Chloro-4-(4-diethylamine-1-methylbutylamine)quinoline] is a cheap, relatively well tolerated drug initially developed for the malaria in the 1930s. CQ inhibits pro-inflammatory cytokine release into human whole blood and may be of therapeutic benefit not only during chronic inflammation, but also in disease that are related to bacteria-induced inflammation.13

CQ was first synthesized in Germany by Hans Andersag in Bayer Corporation in 1934 as a cheaper alternative to the costly naturally occurring quinine. The disadvantages of Quinine/Quinidine are that they are really toxic and have a short half-life.14

CQ is a bitter, colorless, dimorphic crystalline powder soluble in water at pH 4.5. It therefore, dissolves rapidly in the stomach (pH 2.0). CQ has a Quinoline ring like that of quinine and a side chain identical to that of Quinacrine; and the chloride atom in the seventh position appears to be crucial to its antimalarial activity.15

It specifically inhibits the malaria parasite's digestive pathway for hemoglobin.16 There are two enantiomers, the (-)-Chloroquine being less active than (+)-Chloroquine enantiomer against CQ-resistant strains of Plasmodium falciparum.17 Chloroquine acts by binding to heme molecules released from the hemoglobin that is digested by malaria parasites as they grow within their host rbc's. This binding interferes with the process by which heme is normally incorporated into inert crystals and detoxified, thereby effectively poisoning the parasites18 because heme is not a parasite encoded molecule that can mutate under drug pressure, malaria parasites have had to solve the difficult problem of Chloroquine's toxicity by evolving a mechanism either to prevent drug-heme interactions or to control the damage from these complexes. Chloroquine-resistant P. falciparum parasite achieves this by reducing Chloroquine accumulation in their acid digestive vacuoles where the drug does its damage.

Chloroquine resistance (CQ-R) in P. falciparum is associated with mutations in the digestive vacuole of single PfCRT amino acid change K76T from resistant strains leads to wild type levels of CQ susceptibility, increased binding of CQ to its target Ferriprotoporphyrin IX in the digestive vacuole and loss of verapamil reversibility of CQ and quinine resistance. PfCRT mutations preceding residue 76 modulate the degree of verapamil reversibility in CQ-side chain length. Together, these findings establish PfCRT K76T as a critical component of CQR and suggest that CQ access to Ferriprotoporphyrin IX is determined by drug-protein interactions involving this mutant residue.

Mode of Action

Key to the mode of CQ action is the process of hemoglobin degradation, which liberates toxic Ferriprotoporphyrin IX moieties in the intracellular parasite's digestive vacuole (DV). These moieties are detoxified via incorporation into inert hemozoin polymers consisting of FP dimmers. CQ, a weak base, inhibits detoxification by accumulating in the DV as (CQ+2) and binding to FP, causing parasite death presumably as consequence of increased membrane permeability and lipid per oxidation. CQ resistant parasite exhibits elevated CQ IC50 values, reduced CQ accumulation and partial reversibility of resistance by the calcium channel blocker, Verapamil (VP).

The hemoglobin inside the food vacuole is oxidized to methemoglobin in the acidic pH and subsequently hydrolyzed by aspartic proteases into free heme (Fe+3) and denatured globin which is further hydrolyzed to peptides in turn to amino acids which are used by parasite for their protein synthesis. If the relaxed heme is allowed to accumulate within the food vacuole, free heme (Fe+3) level may reach 300-500mM. Free heme is very toxic because it can generate reactive oxygen species and may induce oxidative stress leading to parasite death.

Free heme detoxification systems of Plasmodium falciparum:- The heme detoxification systems can be classified into two: (a) Primary via hemozoin formation in the food vacuole of parasite and (b) Detoxification by GSH, heme binding proteins and degradation of free heme by H2O2.

Major drugs inhibiting hemozoin formation:-

Quinoline and its derivatives:- Important members of this class are Chloroquine, Amodiaquine, Mepacrine, Quinine, Quinidine, and Bisquinoline. Chloroquine is a weakly basic amphipath and accumulated inside the food vacuole. It interacts with μ-oxo dimer form of oxidized heme and prevents the hemozoin formation. Qunine diastereomer, 9-Epiquine also inhibits hemozoin formation by decreasing the rate of hemozoin formation rather than irreversibly blocking its formation. The 7-halo substituted compounds are the most active antimalarials in the 4-aminoquinoline series.

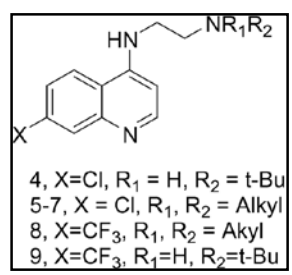
Azoles and its derivatives: - Structure-function relationship: The potent antimycotic drug, Clotrimazole (CLT) inhibits invitro parasite growth of different strains of Chloroquine-sensitive and resistant of P. falciparum (IC50= 0.2-1.1μM).

CLT has a binding affinity for Ferric heme to form a heme-CLT complex, which disturbs heme degradation by GSH and the complex damages the cell membranes more seriously than that free heme does. The CLT-heme complex prevents conversion of free heme into hemozoin. CLT inhibits parasite growth with an IC50 value approximately 1 μM and the full inhibition is achieved at 2.5 μM. Associated with the drug induced hepatotoxicity19 agranulocytosis and life threatening idiosyncratic toxicity seen in humans taking amodiaquine.20 The original lead compound (3), the direct regioisomer of Amodiaquine (2), was selected in order to eliminate para-Aminophenol metabolite alert present in (2) as anticipated, (3) was potent antimalarial, highly active against a broad range of CQ resistant parasite invitro. This subtle modification completely abolished the potential for Quinoneimine formation (catalyzed by either P-450 enzymes or myeloperoxides). Molecule (3) served as a starting point for lead optimization and through an iterative process of synthesis, activity testing, and drug metabolism pharmacokinetic (DPMK) evaluation, the ultimate candidate N-tert-butylisoquine (4) was identified. This molecule confirms to the target product profile (TPP) for an orally active antimalarial as set out by medicines for malaria venture (MMU, Geneva) and the World Health Organization (WHO/TDR). Following demonstration that the N-tert-butyl function of a fluorinated derivative of AQ, (6) was significantly more resistant to metabolic cleavage, we predicted that the N-tert-butyl functional group would provide a significant advantage over N-diethyl amino group present in original lead compound (3). It was hoped that this manipulation would result in a much-simplified metabolic profile and enhanced oral bioavailability.

Result and Discussion: -

Antimalarial activity: Analogue (4) displays excellent low μM activity against CQ-resistant and sensitive parasites with a better antimalarial exposure profile, as measured by the Non-Recrudescence Dose Level (NRL) compared to Amodiaquine or Chloroquine.

Scheme 1



Sidhu et.al. shows that pfcrt alone is sufficient to encode resistance, but this only holds for the genetic background of their selected host strain (50% of which was derived from a CQ-resistant strain). PfCRT Lys76→Thr (K76T) mutations appear to be a prerequisite for parasites to survive during treatment but many of them disappears after drug treatment. Due to dearth of new antimalarials, this has raised fears that cross-reactivity may have evolved because Quinolines and Artemisinin both kill the malaria parasite by interfering with its heme detoxification pathway. Mutations K76T and Ala220→Ser (A220S) appear to be the most reliable markers predicting CQ resistance.

The molecular basis for CQ resistance is not fully understood; however, it is clear that CQ resistant parasites accumulate fewer drugs than sensitive strains.21 In light of these observations, it has been synthesized a new series of CQ analogues where the diethylamino isopentyl function of the side chain has been replaced by shorter side chains, containing metabolically more resilient terminal secondary and tertiary alkyl amino groups.22a The ability of each compound within the series (4-9) (Scheme 1) to arrest the growth of CQ sensitive and CQ resistant strains of P. falciparum in vitro has been measured in the anticipation that these derivatives may circumvent the mechanism of drug resistance. In addition, the ability of these derivatives (4-9) to compete for the same intraparasitic receptor as CQ. Fe (III) FPIX, has been investigated to gain insight into their mode of action and potential mechanism of resistance.22b,23

CONCLUSION

In the battle against malaria, we are currently at a stalemate at best. If and when the resistance to artemisinin drugs further de-

velops and spreads, we will be caught unarmed, without any effective weapon against this deadly parasite. Looking at the antimalarial drug development pipeline, new classes of agents, and, in the meantime, a re-optimization of existing drugs using new creative strategies are needed. Re-optimization of existing drugs through replacement/rotation and combination approaches may prolong their life spans for effective treatment and prophylaxis, until new treatments are found. The future of antimalarial drug discovery lies in innovative thinking and novel areas, some currently under exploration and some yet to explored..

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