

Quinolines and Artemisinin: Chemistry, Biology and History

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Abstract *Plasmodium falciparum* is the most important parasitic pathogen in humans, causing hundreds of millions of malaria infections and millions of deaths each year. At present there is no effective malaria vaccine and malaria therapy is totally reliant on the use of drugs. New drugs are urgently needed because of the rapid evolution and spread of parasite resistance to the current therapies. Drug resistance is one of the major factors contributing to the resurgence of malaria, especially resistance to the most affordable drugs such as chloroquine. We need to fully understand the antimalarial mode of action of the existing drugs and the way that the parasite becomes resistant to them in order to design and develop the new therapies that are so urgently needed. In respect of the quinolines and artemisinins, great progress has been made recently

in studying the mechanisms of drug action and drug resistance in malaria parasites. Here we summarize from a historical, biological and chemical, perspective the exciting new advances that have been made in the study of these important antimalarial drugs.

Abbreviations

AQ	Amodiaquine
CQ	Chloroquine
DHA	Dihydroartemisinin
FP	Ferriprotoporphyrin IX (haematin)
HZ	Haemozoin
MDR	Multi-drug resistance
Mn ^{II} TPP	Manganese tetraphenyl porphyrin
MMV	Malaria for Medicines Venture
PVM	Parasitophorous vacuolar membrane
PPM	Parasite plasma membrane
SAR	Structure activity relationships
SERCA	Sarco/endoplasmic reticulum Ca ²⁺ -ATPase
TCTP	Translationally controlled tumour protein

1

Introduction

Chemotherapeutic strategies for the treatment of *Plasmodium falciparum* malaria have largely relied upon drugs derived from two traditional herbal remedies. Quinine, extracted from Cinchona bark, has provided the basis for the development of synthetic quinoline-containing drugs such as chloroquine (CQ), amodiaquine (AQ) and mefloquine. (Foley and Tilley 1998). More recently, the discovery of artemisinin, extracted from the plant *Artemisia annua*, prompted the design of structural mimics containing a trioxane motif. (Meshnick et al. 1996; Borstnik et al. 2002; Tang et al. 2004) In this chapter, we compare and contrast the quinolines and artemisinins in terms of pharmacological target discovery, mechanism of action and parasite resistance.

It has been known for over 100 years that malaria parasites are susceptible to quinine at the time only when they degrade haemoglobin and produce malarial pigment (Slater 1992) These observations can be extended to all the quinoline-containing drugs and to artemisinins, and subsequent work has amply demonstrated that the processes of haemoglobin ingestion and digestion provide important targets for these classes of antimalarials. For these reasons we begin our review with a summary of the feeding process in *P. falciparum*.

2 The Digestive Apparatus of *P. falciparum*

P. falciparum undergoes cycles of erythrocytic schizogony, which produce the main clinical features of the disease. During an erythrocytic cycle approximately 80% of the host-cell haemoglobin is ingested and is degraded by the developing trophozoite. As the parasite develops, host cell cytoplasm is taken up through the endolysosomal system. A mouth-like structure termed the cytostome is formed from a localized invagination of the parasitophorous vacuolar membrane (PVM) and the parasite plasma membrane (PPM) (Fig. 1). Double-membrane transport vesicles containing host cell cytoplasm are pinched off from the cytostome, (Yayon and Ginsburg 1983). The

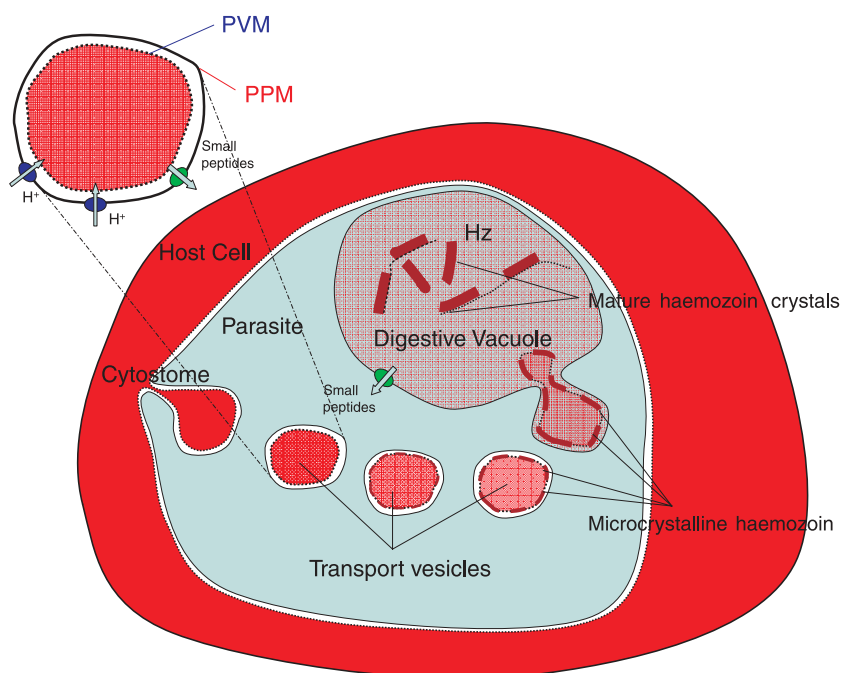


Fig. 1 Schematic showing the parasite feeding apparatus. Host cell cytoplasm is ingested by the cytostome and packaged in double membrane transport vesicles. The inner membrane is derived from the parasitophorous vacuolar membrane (PVM) and the outer membrane is derived from the parasite plasma membrane (PPM). Degradation of haemoglobin may start in the transport vesicles and be completed in the digestive vacuole. FP is deposited on the remains of the PVM which may act as a template for crystal formation. The vesicles fuse with the digestive vacuole and the immature crystals, residual membranes and haemoglobin are delivered to the digestive vacuole

outer membrane of the transport vesicle is formed from the PPM and the inner membrane is formed from the PVM. These vesicles ultimately fuse with the digestive vacuole releasing the PVM-derived vesicle and its contents into the digestive vacuole. During digestion in the endolysosomal system, the protein part of the haemoglobin is broken down, initially into large peptides and later into smaller peptides. This occurs as a result of the concerted action of groups of aspartic and cysteine protease enzymes and a metalloprotease. Digestion is thought to occur in the DV and the process may be initiated in the transport vesicles (Fig. 1). Small peptides are exported from the endolysosomal system, and hydrolysis to free amino acids takes place in the parasite cytoplasm by the action of a cytosolic aminopeptidase.

The ingestion and digestion of most of the host cell haemoglobin is a vast and energetically expensive process for the parasite, involving extensive recycling of its plasma membrane and parasitophorous vacuolar membrane, as well as the generation of a substantial proton gradient that is required for optimum activity of the protease enzymes. Another problem for the parasite is the remaining haem (ferriprotoporphyrin IX, FP) that is released upon digestion of the globin chains. Free FP causes lethal changes to membranes and proteins and its efficient disposal is of critical importance to the parasite. FP is not destroyed enzymatically (as it is in mammalian cells) but instead

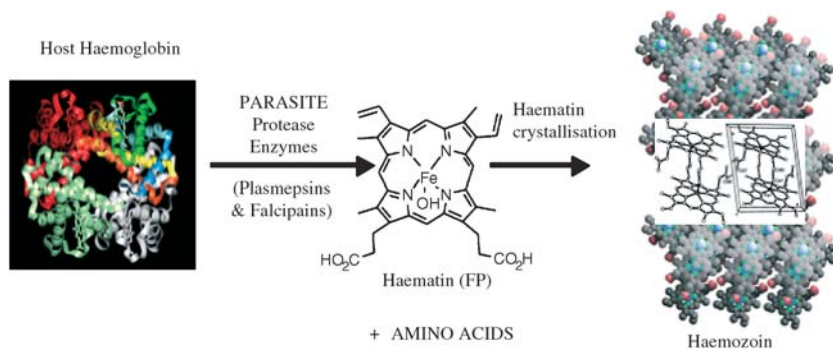


Fig. 2 The haemoglobin degradation pathway and generation of haemozoin. As the parasite matures within its host erythrocyte it digests a large proportion of the host cells' haemoglobin. A number of protease enzymes are involved in this digestion process including aspartic protease (aspartic haemoglobinase I or plasmeppsins I), an enzyme that initiates haemoglobin degradation, whilst a second aspartic protease (plasmeppsins II) cleaves acid denatured haemoglobin. This initial process cleaves haemoglobin to give both FP and globin. The third class of enzymes involved is the cysteine proteases, or falcipains, enzymes that do not recognize haemoglobin or FP, but readily cleave the denatured globin releasing a number of small peptides and amino acids

is converted into a crystalline substance called haemozoin (HZ) or malarial pigment that is harmless to the parasite. The process of HZ formation is of great interest to malariologists, not least because it is thought to be the target of some of the quinoline antimalarials. (Fig. 2).

3

Haemozoin

Until the 1990s, HZ was generally believed to be a hemoprotein but Fitch and Kanjanangkulpan and Slater and co-workers reported that malarial pigment consisted only of FP and essentially resembled β -FP (Fitch and Kanjanangkulpan 1987; Slater et al. 1991), a synthetic FP product that exhibits greatly reduced solubility compared to FP. These authors showed that the infrared spectra of HZ and β -FP, are identical, exhibiting sharp peaks around 1664 and 1211 cm^{-1} (Slater et al. 1991, 1992). These peaks are characteristic of a carboxylate group being coordinated to the iron centre of FP. Further evidence for this iron–carboxylate bond came from the extended X-ray absorption fine structure spectrum indicating the presence of an oxygen atom in the coordination sphere of the iron ion and a carbon atom an appropriate distance away (Slater et al. 1991). These authors also proposed that HZ is polymeric in structure, although no direct evidence for this last proposal was presented. The main thrust of Slater et al. (1991)—that the propionate side chain of one FP coordinates to the iron centre of its neighbour—has been confirmed, but the structure of HZ is not polymeric. In fact, the chemical structure was subsequently shown to be dimeric: one propionate group of each FP moiety coordinates to the iron centre of its partner in the dimer (Pagola et al. 2000). The second propionate group of each FP is hydrogen bonded to a dimer in the neighbouring unit cell.

The exact mechanism of HZ formation is not known (Egan et al. 2001). It is unlikely that the pigment crystals are formed simply by precipitation since they are chemically distinct from the water-soluble species. Direct observation of HZ formation within the parasite is extraordinarily difficult and studies to date have been confined to observing the process of β -FP formation *in vitro*. These studies have largely been conducted using FP in acetate solution, sometimes supplemented with biological material in the form of parasite membrane extract, lipids, purified HZ, parasite proteins or synthetic peptides (for a comprehensive review see Egan 2002). Whilst high concentrations of acetate are clearly non-physiological, such studies can potentially shed some light on the chemical mechanism of HZ formation: it is likely that the formation of synthetic HZ from acetate solution occurs via rapid precipitation of amorphous FP, which is slowly converted to β -FP (synthetic HZ) by acetate

assisted dissolution and re-precipitation (Egan 2002). The rate of nucleation and growth and the crystal morphology are susceptible to manipulation of the reaction conditions (buffer strength, temperature and stirring rate). It was proposed that high concentrations of acetate increase the solubility of FP by forming a negatively charged FP-acetate complex, thus acting as a phase transfer catalyst. A number of studies have shown that β -FP formation can be promoted by lipids and membranes (Bendrat et al. 1995; Fitch et al. 1999, 2000). Intriguingly, it has been suggested that lipids and membranes could play a similar role to acetate in the solubilization of FP for the synthesis of HZ (Hempelmann and Egan 2002; Egan et al. 2001; Egan 2002). Based on the very high affinity of FP for membranes, we recently proposed a hypothesis for the formation of HZ in the parasite. We proposed that the FP (released from the digestion of haemoglobin) partitions into the PVM membrane enclosed within the endocytic vesicles (Fig. 1; Hempelmann et al. 2003). This has several consequences. Firstly, the PVM acts as a local 'sink' for FP and protects the membranes of the surrounding endocytic vesicles from the damaging effects of FP. Secondly, the hydrophobic environment of the PVM provides conditions that favour the formation of the iron-carboxylate bond of the β -FP dimer (Egan et al. 2001); Formation of this bond in aqueous solutions has high activation energy, requiring non-physiological conditions of temperature or ionic strength in order to grow HZ crystals in vitro (Egan 2002). Thirdly, the PVM provides a 'scaffold' or template for the growing HZ crystals.

Other investigators believe that the transport vesicles are for delivery only and that haemoglobin digestion is confined to the central food vacuole (Yayon and Ginsburg 1983, 1984). There is no definitive evidence to support either case and it is important to emphasize that the PVM remnants are present at the site of haemoglobin digestion and may play the same important role in HZ production, regardless of which hypothesis is correct.

4

Quinolines

4.1

Mode of Action of Quinoline-Containing Drugs

Following the isolation of quinine (3) from the bark of the Cinchona tree by Pelletier and Caventou in 1820, this compound was used for the treatment of malaria until the 1930s (Foley and Tilley 1998). Since then chloroquine, (1) and other synthetic quinoline antimalarials [AQ (2) and mefloquine (4); Fig. 3] have been mainstays of malaria chemotherapy for much of the past 50 years (Foley and Tilley 1998; O'Neill et al. 1998). The key to the success

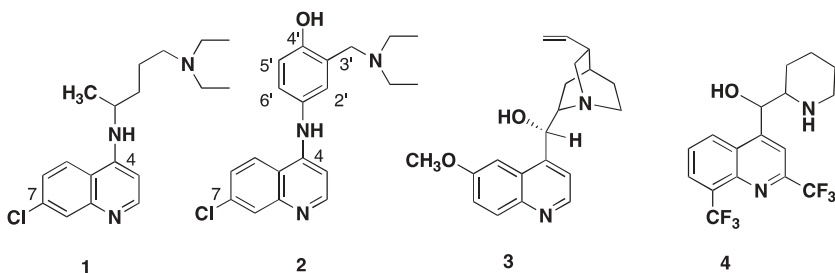


Fig. 3 Quinoline antimalarials: structures of chloroquine (1), amodiaquine (2), quinine (3) and mefloquine (4)

of the most important synthetic quinoline, CQ has been the excellent clinical efficacy, limited host toxicity, ease of use and simple, cost-effective synthesis. However, the value of synthetic quinoline based antimalarials has been seriously eroded in recent years, mainly as a result of the development and spread of parasite resistance (Winstanley et al. 2002; Warhurst 2001). Although much of the current effort is directed towards the identification of novel chemotherapeutic targets, we still do not fully understand the mode of action of and the mechanism of resistance to both the 4-aminoquinolines (CQ) and the quinoline methanols (quinine, mefloquine), knowledge that would greatly assist the design of novel, potent and inexpensive quinoline antimalarials.

The exact mode of action of CQ remains to be elucidated but most investigators now accept that a critical step in this process is the binding of the drug to FP. The antimalarial activity of CQ stems directly from its highly selective uptake and concentration in malaria-infected erythrocytes (Homewood et al. 1972; Diribe and Warhurst 1985; Fitch et al. 1974; Verdier et al. 1985; Hawley et al. 1998). This could be due in part to a proton trapping mechanism: CQ is a weak base that in its uncharged form will diffuse freely into acidic compartments where it binds to protons and becomes trapped (Homewood et al. 1972; Yayon et al. 1985). However, CQ accumulation by malaria parasites is at least 20-fold higher than its accumulation by mammalian cells which also possess large acidic lysosomes (Hawley et al. 1996) and we have shown that the rate and extent of CQ uptake is largely determined by the concentration of free FP in the parasite (Bray et al. 1998, 1999). The steps following the initial binding of CQ to FP and ultimately leading to parasite death are less clear. Fitch and colleagues have proposed that CQ-FP complex accumulates in parasite membranes, ultimately destroying the membranes by a lipid peroxidation mechanism and killing the parasite by lysis (Fitch 1982). Other investigators believe that CQ works by inhibiting the formation of HZ crystals, and that this leads to the build up of toxic concentrations of free FP within the parasite

(Goldberg and Slater 1992; Slater and Cerami 1992; Sullivan 2002). The way in which free FP is supposed to kill parasites is not always explicitly stated but it could involve inhibition of parasite feeding by inhibiting critical parasite enzymes such as digestive vacuole proteases (van DerJagt et al. 1987). Further complications arise upon consideration that inhibition of HZ crystal formation can be mediated by at least two distinct mechanisms: substrate depletion caused by CQ binding (Dorn et al. 1998a, 1998b) or by direct binding to and capping of the HZ crystal by CQ (Sullivan 2002). It is not known which of these mechanisms (if any) is operating in the parasite.

Many studies have shown that CQ and similar drugs inhibit the formation of HZ crystals in vitro and it has been shown that CQ can bind directly to HZ (Sullivan 2002). However, there is no direct evidence that inhibition of HZ formation is actually responsible for killing the parasite. Most investigators would agree that parasites are killed by a build-up of toxic FP or FP-CQ complex. However, none of the studies published so far are able to discriminate between a direct build-up of CQ-FP complex and a build-up of FP/FP-CQ complex as a secondary effect following the inhibition of HZ formation. Nonetheless, the haemoglobin degradation pathway in *P. falciparum* is a specialized parasite process with a proven history as an exploitable therapeutic target. Unlike parasite encoded enzymes that are currently under investigation, the parasite has difficulty in developing resistance to drugs that bind FP (compare the speed of resistance development to CQ with that for the antifolates or atovaquone; McKeage and Scott 2003). This is because the CQ-resistant parasite is apparently unable to alter the quantity or nature of the FP target (Zhang et al. 1999). Furthermore, in view of the ease of developing new derivatives that avoid the CQ resistance mechanism (see below), the development of new 4-aminoquinoline derivatives remains a valid strategy.

Other quinoline-containing drugs are thought to act in a similar way to CQ in that they all bind to FP (Munghthin et al. 1998; Sullivan 2002; Foley and Tilley 1998). However, their effects on the feeding process seem to be subtly different. For instance, it has been shown that CQ and other 4-aminoquinolines cause a build up of undigested haemoglobin in the parasite (Yayon et al. 1984) whereas quinine and mefloquine do not (Famin and Ginsburg 2002). It was suggested that whereas CQ and AQ inhibit the digestion of haemoglobin, mefloquine and quinine inhibit the ingestion of host cell haemoglobin, possibly by interfering with the endocytic process.

Since the interaction with haematin (ferriprotoporphyrin IX, FP) in the digestive vacuole of the parasite appears to be key to the mechanism of action of 4-aminoquinolines (Foley and Tilley 1998), several studies have attempted to probe the effect of chemical substitution on this interaction. Vipagunta and co-workers employed isothermal titration calorimetry studies to derive association constants for CQ-FP binding at neutral pH (Vipagunta et al. 2000).

From this work it was shown that CQ binds to two μ -oxo dimers in a sandwich arrangement originally proposed by Moreau (Moreau et al. 1982, 1985). Importantly, these studies demonstrate that CQ–FP binding is independent of ionic strength suggesting that an interaction between the charged side-chain terminal nitrogen of CQ with the carboxylates of FP may not play a major role in complex formation. From this study the importance of the chlorine atom at the 7-position of the quinoline ring was underlined. Remarkably, Vippagunta et al. were not able to measure any significant interaction between FP μ -oxo dimer and the 6-chloro analogue of CQ (Vippagunta et al. 1999). This result indicates that the 7-chloro substituent in CQ is a critical structural determinant in its binding affinity to FP μ -oxo dimer. Molecular modelling experiments reinforced the view that the enthalpically favourable π – π interaction observed in the CQ–FP μ -oxo dimer complex derives from a favourable alignment of the out-of-plane π -electron density in CQ and FP dimers at the points of intermolecular contact. For 4-aminoquinolines related to CQ, these data suggests that electron-withdrawing functional groups at the 7-position of the quinoline ring are required for activity against both FP crystallization and parasite growth and that chlorine substitution at position 7 is optimal. Kashula, Egan and co-workers who used 40% DMSO/water, conditions that maintain FP in a monomeric state, backed up this observation regarding the 7-position of the quinoline nucleus (Kaschula et al. 2002). Stoichiometries of CQ binding to FP were recorded to be 1:1 in these studies and it was shown that complexation with FP monomer correlated with the electron withdrawing capacity of the atom at the 7-position (using Hammett constants). A direct relationship between antimalarial activity normalized for pH trapping and antimalarial activity was also clearly demonstrated in this study (Kaschula et al. 2002).

Since it is still not clear whether CQ interacts primarily with FP monomers, μ -oxo dimers or other forms of FP in the food vacuole, for simplicity we recently carried out modelling studies on a 1:1 complex of CQ–FP in line with our previous work (O'Neill et al. 1997). We also note that other works have suggested that CQ may inhibit HZ formation by blocking the growing face of the HZ crystal by a capping effect (Buller et al. 2002; Chong and Sullivan 2003). In this case and in the case of interactions with a monomer, since the bonding interactions are co-facial, we would anticipate that the interaction energies involved in complexation may be similar.

From our earlier work (O'Neill et al. 1997) (we suggested that the principle bonding interactions between FP and CQ (and AQ) were π – π stacking interactions of the quinoline ring over the porphyrin with a possible additional weak electrostatic interaction between the protonated ammonium function and the carboxylate groups of FP. We could find no evidence, in this earlier work, to support an H-bonding interaction between the protonated nitrogen of CQ or AQ and the carboxylates of the FP side chain. In comparison to CQ, shorter

2-carbon chain analogues of CQ (*vide infra*) have a significantly reduced distance for the protonated charged ammonium nitrogen to the carboxylates in the FP-drug complex ($<2.80 \text{ \AA}$). As shown in Fig. 4, modelling suggests that for a synthetic analogue F2Bu, the principal interactions are π - π stacking of the quinoline ring with an additional potential for an interaction of the protonated ammonium function with the FP carboxylates (F2Bu, 2.6 \AA for NH to O in FP) (Stocks et al. 2002; Fig. 4). With further appropriate isothermal titration calorimetry experiments on these short chain analogues, it remains to be seen whether the principal interactions in the FP-drug complex are the same as those in the CQ-FP complex with an absence of electrostatic interactions as suggested in these modelling studies. Nuclear magnetic resonance (NMR) studies of aqueous solutions of FP μ -oxo dimers with CQ, quinine and quinidine have recently been performed (Leed et al. 2002). Based on the influence of paramagnetic Fe(III) on the relaxation rate of nearby protons of the drug in the complex, it has been possible to measure precise atomic distances in the complex. This information was then used in distant restraint molecular modelling to provide solution structures for unprotonated, protonated and diprotonated complexes of CQ with the FP- μ -oxo dimer. The results suggest a role for the aliphatic side chain of CQ in stabilizing the complex and that the positioning of the quinoline ring over the face of the iron porphyrin was towards the edge of the tetrapyrrole ring rather than above the ferric iron centre (Moreau et al. 1982, 1985). This is in line with the known and favourable edge-to-face arrangement for π - π stacking interactions. The same approach

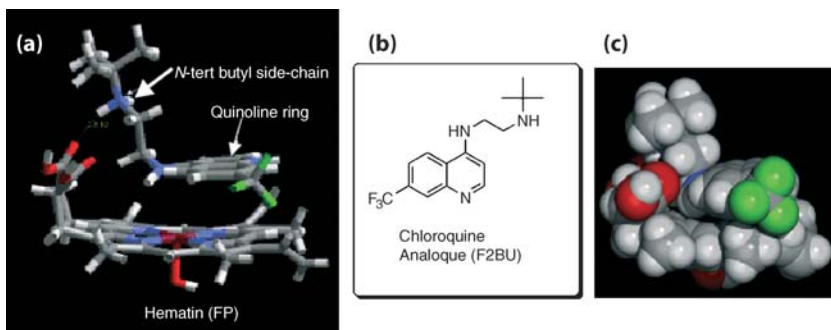


Fig. 4a–c Molecular modelling of the FP–quinoline drug interaction. The figure depicts a low-energy complex of haem [Fe(III)FPIX] (in vacuo) with a synthetic quinoline F2TB and clearly shows the π - π -stacking interaction between the porphyrin ring of FP and the quinoline ring and an electrostatic interaction between the carboxylic acid group and the protonated amino side chain of the drug. The space-filling model for the complex is shown on the right. c Carbon atoms are represented in grey. Hydrogens are white, nitrogens are blue, oxygens light red, chlorine green and iron dark red

was also applied to FP μ -oxo dimers and quinine and quinidine but will not be discussed here and the reader is referred to the original publication (Leed et al. 2002).

4.2

Resistance to Quinoline-Containing Drugs

One of the most striking characteristics of CQ is its capacity to concentrate in the digestive vacuole of the trophozoite. It is here that it forms complexes with FP and inhibits haemoglobin degradation (see above). It has long been known that CQ-resistant isolates exhibit reduced accumulation of CQ when compared with their CQ-sensitive counterparts (Fitch, 1969, 1970; Verdier et al. 1984, 1985). More recently, we have shown that reduced CQ accumulation is manifest as a reduced apparent affinity of CQ-FP binding in the food vacuole of CQ-resistant isolates (Bray et al. 1998). It is evident that CQ-resistant isolates have evolved a mechanism (most likely a transporter or change in pH gradient) to reduce the access of CQ to FP (Bray et al. 1998). Another well documented property of CQ-resistant isolates is the ability of verapamil to stimulate the accumulation of CQ, make the parasite sensitive to CQ and effectively 'reverse' the resistant phenotype (Martin et al. 1987; Krogstad et al. 1987; Bray and Ward 1998). Verapamil was shown to act by increasing the access of CQ to the FP receptor (Bray et al. 1998). The verapamil effect is widely accepted as an essential phenotypic marker of CQ resistance. Recent groundbreaking work from the Welles and Fidock laboratories has identified PfCRT (Fidock et al. 2000) an integral digestive vacuole membrane protein that appears to be the determinant of CQ-resistance. Specific polymorphisms of this gene are found in all natural isolates from clinical CQ treatment failures (Wernsdorfer and Noedl 2003) and in vitro in isolates with a CQ-resistant phenotype. Allelic exchange experiments have proven that the characteristic phenotypic markers of CQ resistance (reduced CQ sensitivity, reduced CQ uptake and verapamil effect) can be unambiguously attributed to these specific amino acid changes in PfCRT (Fidock et al. 2000; Sidhu et al. 2002). Being situated on the digestive vacuole membrane, PfCRT is in the right place to influence the access of CQ to FP but at the moment there is no definitive evidence to support any particular mechanism. Two main theories have been proposed: an indirect mechanism in which PfCRT is involved in vacuolar pH homeostasis and a mechanism in which mutant PfCRT directly transports CQ out of the digestive vacuole. The pH hypothesis proposes that reduced vacuolar CQ accumulation arises as a consequence of a primary effect of the PfCRT mutations in reducing the resting pH of the digestive vacuole (Ursos and Roepe 2002). In a mechanistic explanation the authors suggested that reduced vacuolar pH increases the rate of aggregation of FP as it is released from haemoglobin, reducing the

amount that is available to bind CQ. However, this scenario would reduce the number of binding sites rather than reducing the apparent affinity of binding and therefore is not consistent with the experimental observations (Bray et al. 1998). Neither is it consistent with the complex patterns of cross-resistance that are observed between CQ and other quinoline antimalarials. Quinoline drugs which are all weak bases and all bind to FP should be influenced in the same qualitative way as CQ by a gross change in vacuolar pH (Cooper et al. 2002). By contrast, the transport hypothesis is consistent with the available data. For instance, it is easy to envisage subtle changes in drug structure conferring very different affinities for a drug transporter and very different patterns of sensitivity. The transporter hypothesis is also consistent with the reduced apparent affinity of FP binding that is observed in CQ-resistant isolates. Thus it is likely that mutant PfCRT is acting as a drug transporter although formal proof will require further experiments.

The original observations of CQ-resistance reversal were reminiscent of the verapamil reversal of multi-drug resistance (MDR) in certain cancer cell lines (Martin et al 1987; Ambudkar et al. 1999). In this case, the MDR phenomenon is attributable to massive amplification of the *mdr1* gene. This leads to overexpression of the protein product, P-glycoprotein, on the plasma membrane of the cell. P-glycoprotein is a primary active transporter of the ATP-binding cassette superfamily. It is polyspecific, being capable of pumping a broad range of hydrophobic, amphiphilic drugs and xenobiotics out of the cell (Ambudkar et al. 1999). A search for P-glycoprotein orthologues in *P. falciparum* identified Pgh1, an integral digestive vacuole membrane protein of the ATP-binding cassette superfamily (Wilson et al. 1989). Specific amino acid changes in Pgh1 undeniably do modulate the susceptibility of parasites to CQ (Reed et al. 2000) but overall, it would be fair to say that this protein probably plays only a minor role in determining parasite resistance to this drug. However, it is becoming abundantly clear that Pgh1 is very important in determining the response of parasites to mefloquine and other quinolines as well as to artemisinins (Reed et al. 2000). Allelic exchange experiments have shown that mutations in Pgh1 can confer resistance to mefloquine, quinine and the structurally related drug halofantrine (Reed et al. 2000). The same mutations were also able to influence the level of susceptibility to artemisinin and CQ (Reed et al. 2000). More recently, it has been shown that a reduced response of clinical isolates to mefloquine and to artemisinin is strongly correlated to the copy number of the *PfMDR1* gene and to expression of Pgh1 (Uhlemann et al. 2004; see the chapter by A.-C. Uhlemann and S. Krishna, this volume).

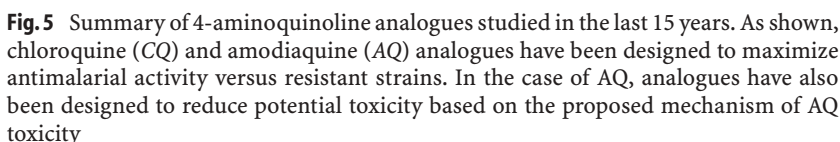
Amodiaquine (2) is a 4-aminoquinoline antimalarial that is effective against many CQ-resistant strains of *P. falciparum*. However, clinical use has been severely restricted because of associations with hepatotoxicity and

agranulocytosis when used in prophylaxis (Jewell et al. 1995). The AQ side-chain contains a 4-aminophenol group; a structural alert for toxicity, because of metabolic oxidation to quinoneimines. We have shown that AQ does indeed readily undergo oxidation to a quinoneimine. (Naisbitt et al. 1998; Tingle et al. 1995).

Figure 5 summarizes the different classes of 4-aminoquinoline that have been investigated over the past 15 years (O'Neill et al. 1998). Because AQ retains antimalarial activity against CQ-resistant parasites our initial studies involved the design and synthesis of fluoroamodiaquine (**4a**) as a safer alternative to AQ (O'Neill et al. 1994). This analogue cannot form toxic metabolites by P450-mediated processes and retains substantial antimalarial activity versus CQ-resistant parasites. Lead optimization of (**4a**) produced a new lead, compound (**4b**) which is about half as active as AQ against CQ-resistant strains in vitro, but shows equivalent oral in vivo potency versus *P. berghei*. Concern about cost led to the consideration of three other series of synthetically more accessible analogues—the tebuquine series (**5**) (O'Neill et al. 1996, 1997) the bis-Mannich series (**6**) (Barlin et al. 1993; Kotecka et al. 1997) and the 5'-alkyl series class of 4-aminoquinoline (**7**) (Raynes et al. 1999). Compounds in the tebuquine and bis-Mannich series have now been shown to have unacceptable toxicity profiles and extremely long half-lives (Ruscoe et al. 1998). Recently, Delarue et al. have prepared some 4'-dehydroxy analogues, some of which have very good activity profiles and are less toxic than AQ. (Delarue et al. 2001).

Studies on 4-aminoquinoline structure–activity relationships (SAR) have revealed that 2-carbon side-chain CQ analogues retain activity against CQ-resistant *Plasmodium* (De et al. 1997; Ridley et al. 1996). Our own efforts (Stocks et al. 2002) were directed towards compounds less likely to undergo metabolic N-terminal dealkylation, a process that produces N-desalkyl metabolites that are considerably less potent against CQ-resistant strains. Some of these 2-C analogues, e.g., (**9**), display good antiparasitic profiles. Other notable work in the CQ–SAR field has involved the preparation of bisquinoline dimers, some of which possess excellent activity against CQ-resistant parasites (Vennerstrom et al. 1998). Unfortunately, the best candidates (example WR-268, 668) to emerge from this series of analogues were shown to be photosensitizing.

From our SAR studies in the AQ series, it is clear that the presence of a 4-aryl amino moiety provides analogues with superior activity against CQ-resistant strains and that the presence of an aromatic hydroxyl function appears to be important for additional levels of antiparasitic activity. We reasoned that interchange of the 3'-Mannich side chain with the 4'-OH function would provide a new template, chemically incapable of forming potentially toxic quinoneimine metabolites. Furthermore, these compounds should be as cheap to prepare as AQ on an industrial scale. Our initial studies show that



To conclude, 4-aminoquinoline-based drug development projects continue; at least three projects are approaching evaluation in man, including short chain CQ analogues, metabolically stable AQ analogues and aza-acridine derivatives (pyronaridine). It is anticipated that successful development of any of these candidates will provide the same sort of therapeutic benefits provided by CQ in its early pre-resistance days.

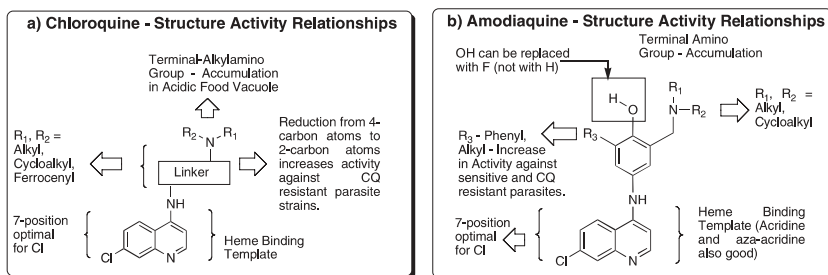


Fig. 6a,b Summary of important SAR observations in the chloroquine and amodiaquine classes

5 Artemisinins

Artemisinin (11a) (qinghaosu) is an unusual 1,2,4-trioxane that has been used in China for the treatment of MDR *P. falciparum* malaria but its therapeutic value is limited by its low solubility in both oil and water (Fig. 7). Consequently, in the search for more effective and soluble drugs, Chinese researchers prepared a number of derivatives of the parent drug. (Klayman 1985; Butler and Wu 1992) Reduction of artemisinin produces dihydroartemisinin (11b), which has in turn led to the preparation of a series of semisynthetic first-generation analogues which include artemether (11c, $R = -\text{Me}$) and arteether (11d, $R = -\text{Et}$) (Fig. 7) (Butler and Wu 1992). Both of these compounds are more potent than artemisinin but have short plasma half-lives and produce fatal central nervous system (CNS) toxicity in chronically dosed rats and dogs (Brewer et al. 1994). Although neurotoxicity is an issue in animal models, recent studies by White and co-workers have shown a lack of neuronal death in patients who had received high doses of artemether by intramuscular injection (Hien et al. 2003). In spite of these observations, there are no

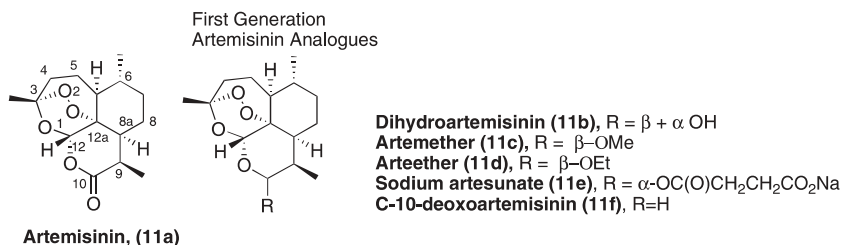


Fig. 7 Structures of artemisinin and the first-generation 'semi-synthetic peroxides'

comparative data on oral dosing with first generation alkyl ether pro-drugs (i.e., artemether, arteether) of the neurotoxic dihydroartemisinin.

For treatment of advanced cases of *P. falciparum* malaria, a water-soluble derivative of artemisinin is desirable. A water-soluble derivative can be injected intravenously (i.v.), (Lin et al. 1989). The sodium salt of artesunic acid (**4e**) is such a water-soluble derivative, capable of rapidly diminishing parasitaemia and restoring consciousness of comatose cerebral malaria patients (Lin et al. 1989). Due to the high recrudescence rate, however, sodium artesunate (**4e**) is normally administered in combination therapy, most often with mefloquine for treatment of uncomplicated malaria (Barradell and Fitton 1995). Of the first-generation derivatives, sodium artesunate (**4e**) is currently the drug of choice, but the combination of artemether and lumenfantrine produced by Novartis is being implemented as part of the artemisinin combination therapy and is likely to see more widespread use.

5.1

Mechanism of Activation

5.1.1

Carbon Radicals, Open Hydroperoxides and High-Valent Iron-Oxo Species: Reactive Species Implicated in the Mechanism of Action of the Artemisinins

The key pharmacophore in artemisinin is the 1,2,4-trioxane unit and, in particular, the endoperoxide bond is crucial for expression of antiparasitic activity. Reduction of the peroxide bridge of artemisinin to give the analogue deoxoartemisinin results in a complete abolition of antimalarial activity (Klayman 1985; O'Neill and Posner 2004). Based on the seminal work of Posner and co-workers in the early 1990s, the free-radical chemistry of artemisinin is now very well defined and has been shown to involve an initial chemical decomposition induced by haem Fe(II) (reduced haemin) or other sources of ferrous iron within the malaria parasite to produce initially an oxy radical that subsequently rearranges into one or both of two distinctive carbon-centred radical species (Posner and Oh 1992; Posner et al. 1994; Posner and O'Neill 2004). Figure 8 summarizes the main radical pathways available for artemisinin following endoperoxide-mediated bioactivation. Since artemisinin is an unsymmetrical endoperoxide, the oxygen atoms of the peroxide linkage can associate with reducing ferrous ions in two ways. Association of Fe(II) with oxygen-1 provides an oxy radical that goes on to produce a primary carbon-centred radical (**12a**). A surrogate marker for the intermediacy of this radical species is the ring-contracted tetrahydrofuran product **12b**. Alternatively, association with oxygen 2 provides an oxy radical species, that, via a 1,5-H shift, can produce a secondary carbon-centred radi-

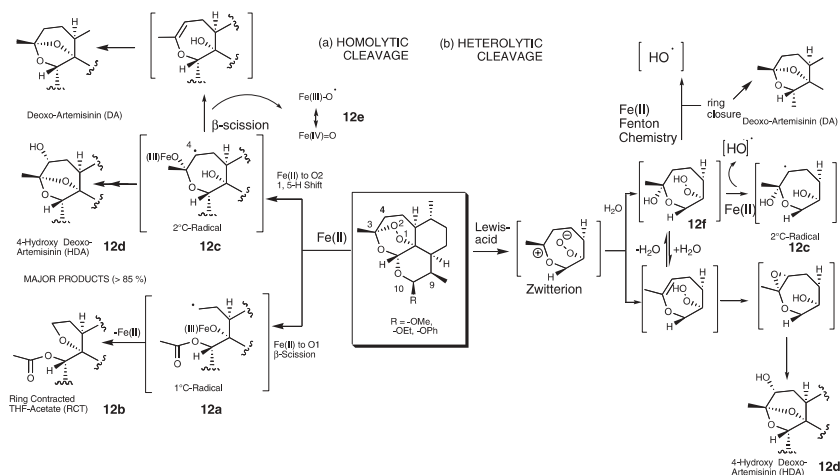


Fig. 8a,b Homolytic and heterolytic mechanisms of bioactivation of the endoperoxide bridge of artemisinin and derivatives

cal (12c). Again, like the previous route, a stable end-product, hydroxydeoxy artemisinin (12d) functions as a surrogate marker for this secondary carbon centred radical species.

There is evidence to support the roles of each individual carbon radical species as the mediators of antimalarial activity and this subject remains an area of intense debate (Posner and Meshnick 2001; Haynes et al. 2003b; O'Neill and Posner 2004; Haynes 2001; Olliaro et al. 2001b). It has been proposed that final alkylation by these reactive carbon radical intermediates of biomacromolecules such as haem, specific proteins and other targets, result in the death of malaria parasites.

The secondary C-radical intermediate (12c) has also been implicated as the precursor to a high-valent iron oxo species (12e), and several experimental results support the intermediacy of such a potentially toxic species (Posner et al. 1995, 1996). Although Varotsis has provided Raman spectroscopic support for the generation of a high-valent iron-oxo species during ferrous-mediated endoperoxide decay (Kapetanaki and Varotsis 2000, 2001) the groups of Meunier (Robert and Meunier 1998) and Jefford (Jefford 2001; Jefford et al. 1996) have contested this chemical mechanism.

Definitive evidence for the generation of carbon radical intermediates during ferrous-mediated endoperoxide degradation of both artemisinin (Wu et al. 1998) and arteflene (O'Neill et al. 2000) has been provided by electroparamagnetic resonance spin-trapping techniques (Butler et al. 1998). For artemisinin, both the primary and secondary carbon centered radicals have

been efficiently spin-trapped post iron-mediated activation (O'Neill et al. 2000; Wu et al. 1998).

An alternative view to the iron-induced homolytic endoperoxide cleavage hypothesis is that artemisinin acts as a masked source of hydroperoxide (Scheme 2, b). (Haynes et al. 1999; Haynes and Vonwiller 1996a, 1996b) Following specific non-covalent interactions with a given target protein, heterolytic cleavage of the endoperoxide bridge and formation of an unsaturated hydroperoxide is followed by capture by water (or other nucleophile). This process provides a reactive hydroperoxide capable of irreversibly modifying protein residues by direct oxidation.

Fenton like degradation of this hydroperoxide may produce the hydroxyl radical, a species that can go on to oxidize target amino acid residues: this alternative pathway provides a mechanism of producing a whole host of reactive oxygen species that may have an equally important role to play in the antimalarial activity of these compounds. It has been proposed that the heterolytic step is aided by the non-endoperoxide bridging oxygen of the trioxane ring, where the carbocation can be stabilized by resonance (Haynes et al. 1999; Olliaro et al. 2001a). Haynes and co-workers have provided direct chemical evidence for this mechanism of bioactivation by demonstrating that artemisinin can mediate *N*-oxidation of tertiary alkylamine derivatives via the intermediacy of such a ring opened peroxide form of artemisinin (Haynes et al. 1999).

It is clear from the above discussion that artemisinin and endoperoxide-based drugs have the ability to generate a range of different reactive intermediates, and many of these have been proposed as the mediators of the phenomenal antimalarial activity of this class of drug.

5.2

The Biological Target(s) of Artemisinin Derivatives

Like the quinolines many hypotheses to explain mechanism of artemisinin action have been proposed. Proposed targets of these species are discussed and will conclude with the most recent studies by Eckstein-Ludwig who suggest that artemisinin derivatives target PfATPase6, the sarco/endoplasmic reticulum Ca^{2+} -ATPase (SERCA) of the parasite. Since the O–O bond appears to be crucial, the haemoglobin degradation pathway of malaria parasites would appear to offer the next clue as to the mechanism of action of the artemisinin class of endoperoxide. Since the original proposal by Meshnick and co-workers (Meshnick et al. 1991) it is still believed by many researchers in the field that haem liberated in this proteolysis process (ferrous haem is produced from 1 electron reduction of oxidized ferric FP) is the species responsible for the bioactivation of the endoperoxide bridge to potentially toxic free radicals in the food vacuole of the parasite (see above).

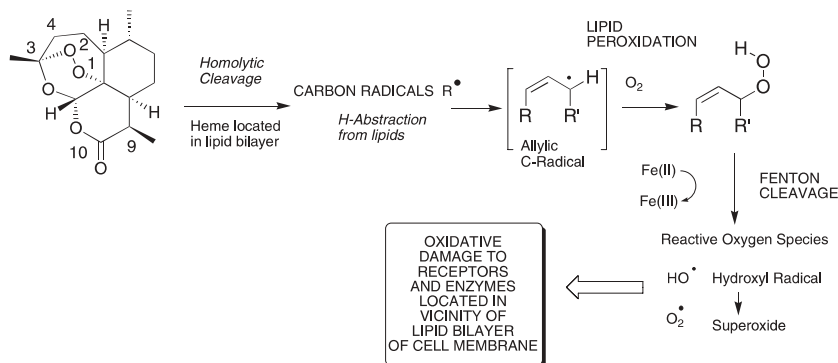


Fig. 9 Proposed chemical mechanism for artemisinin mediated lipid peroxidation of membranes

Consistent with this proposal, biomimetic studies by Berman and Adams have clearly demonstrated that artemisinin can effect a sixfold increase in haem-mediated lipid membrane damage (Berman and Adams 1997). Of importance to this observation are the findings of Fitch and colleagues who have recently demonstrated that unsaturated lipids co-precipitate with FP in the parasite's acidic food vacuole and also dissolve sufficient monomeric FP to allow efficient crystallization (Fitch et al. 1999). A possible mechanism of artemisinin induced lipid peroxidation is depicted in Fig. 9 and provides downstream access to typical reactive oxygen species such as oxyl radicals and the superoxide anion. Interaction of lipid solubilized haem with artemisinin followed by ferrous-mediated generation of oxyl and carbon radicals places these reactive intermediates in the vicinity of target allylic hydrogens of unsaturated lipid bilayers. Hydrogen abstraction and allylic carbon radical formation with subsequent triplet ground state oxygen capture results ultimately in the formation of lipid hydroperoxides. The explicit mechanism depicted in Fig. 9 is supported by the work of Berman and Adams (1997) and others and it was proposed that the damage caused to the parasite's food vacuole membrane leads to vacuolar rupture and parasite autodigestion. The biological significance of hydroperoxides in relation to biological hydroxylation and autoxidation of, for example, lipids and membrane bilayers, is well established. The generation of unsaturated lipid hydroperoxides provides a means of initiation of such processes.

In contrast to these proposals, other workers in the field have suggested that membrane bound haem may have a role to play in reducing the effectiveness of endoperoxides such as dihydroartemisinin (Vattanaviboon et al. 2002). Further work is required to clarify the role of vacuolar membrane bound haem in the mechanism of action of endoperoxide antimalarials.

Although the above scheme would appear to be chemically plausible, several workers have proposed that parasite death in the presence of artemisinin is probably not due to non-specific or random cell damage caused by freely diffusing oxygen radical species, but might involve specific radicals and targets some of which are described below (Robert and Meunier 1998; Robert et al. 2002).

When artemisinin or other active trioxanes were incubated at pharmacologically relevant concentrations within human red blood cells infected by *P. falciparum*, a haem-catalysed cleavage of the peroxide bond was reported to be responsible for the alkylation of haem (Zhang et al. 1992) and a small number of specific parasite proteins, (Yang et al. 1993, 1994) one of which has a molecular size similar to that of a histidine-rich protein (42 kDa). Another possible target protein is the *P. falciparum* translationally controlled tumour protein (TCTP). In vitro, dihydroartemisinin reacts covalently with recombinant TCTP in the presence of haemin. The association between drug and protein increases with increasing drug concentrations until it reaches a stoichiometry of 1 drug molecule/TCTP molecule. The function of TCTP is unknown and thus the role of haemin-mediated artemisinin alkylation in the mode of action of this drug awaits further biochemical definition (Bhisutthibhan et al. 1998; Bhisutthibhan and Meshnick 2001). Artemisinin may also be involved in the specific inhibition of malarial cysteine protease activity (Hong et al. 1994; Pandey et al. 1999).

5.2.1

Haem and Haem Model Alkylation

Alkylation of haem by artemisinin was first reported by Meshnick after identification of haem-drug adducts by mass spectrometry, but no structures were proposed for the resulting covalent adducts (Hong et al. 1994; Zhang et al. 1992). Because of the variety of possible alkylation sites on iron protoporphyrin-IX, Meunier studied the alkylating activity of artemisinin with manganese(II) tetraphenylporphyrin, a synthetic metalloporphyrin having a fourth-order symmetry and only the eight equivalent β -pyrrolic positions as possible alkylation sites. By reacting manganese tetraphenyl porphyrin (Mn^{II} TPP) with artemisinin (or artemether and several related synthetic trioxanes) in dichloromethane, a chlorin-type adduct was formed by reaction of the macrocycle with an alkyl radical generated by reductive activation of the drug endoperoxide (Robert et al. 1997).

Further studies involved the investigation of the reactivity of artemisinin toward the pharmacologically more relevant iron(II) containing model of ferriprotoporphyrin IX (Robert et al. 2001). For this purpose, iron(III) protoporphyrin-IX dimethylester was exposed to artemisinin in the presence

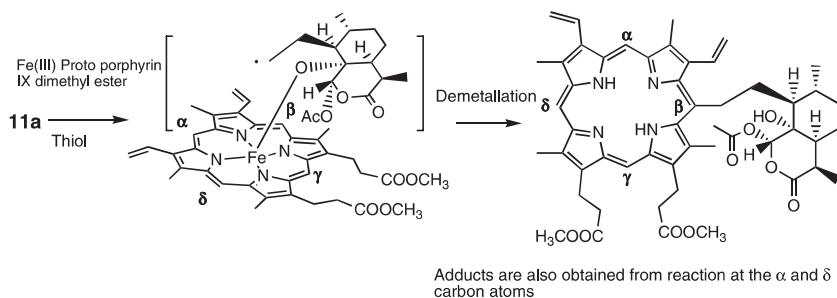


Fig. 10 Alkylation of a haem model (the ester of FP) by a primary carbon centred radical derived from bioactivation of artemisinin

of a hydroquinone derivative (or a thiol), as reducing agent, to generate the requisite iron(II) haem species. Haem was readily converted in high yield to haem–artemisinin adducts (Fig. 10; Cazelles et al. 2001). After demetallation of this mixture of three adducts to facilitate the NMR characterization, indications were that the α , β and δ meso carbons were alkylated; such results prompted Meunier to suggest that the low and transient concentration of free haem generated by haemoglobin degradation in vivo may be responsible for the reductive activation of the endoperoxide function of active trioxanes. This pathway generates alkylating species, such as the primary carbon radical (12a), which are likely to disrupt vital biochemical processes of the parasite via alkylation of biomolecules located in the close vicinity of the free haem (Robert and Meunier 1998). This proposal is based on the assumption that the primary C-radical has sufficient life-time to migrate from the face of the porphyrin metallocycle and subsequently to interact with its biological target (Olliaro et al. 2001a).

Although it is clear in model systems that artemisinin can efficiently alkylate haem-based models, the role of this event in the mechanism of action of artemisinin has been questioned. For example, it has been proposed that the formation of haem–artemisinin adducts of the type described could result in the prevention of haem crystallization to non-toxic HZ. The resultant build up of redox active alkylated porphyrins could in theory lead to parasite death by a mechanism similar to that proposed for the quinoline-based antimalarials. However, Haynes and co-workers have ruled out this potential mechanism by demonstrating clearly that although artemisinin (11a) and dihydroartemisinin (11b) have the ability to inhibit beta-FP formation in vitro, the closely related and antimalarially potent C-10 deoxy artemisinin (11f) (Fig. 7) has no effect on crystallization (Haynes et al. 2003c). Thus, it was proposed that the observed inhibitory activities in the haem polymerization inhibitory assay (HPIA) for 11a and 11b are a reactivity or property not related

to the inherent antimalarial mode of action of this class of drugs (Haynes et al. 2003c).

It should be emphasized that virtually all of the above discussion is based on biomimetic chemistry where the Fe(II) source varies from salts such as FeSO_4 to the more reactive $\text{FeCl}_2 \cdot 4\text{H}_2\text{O}$ as well as haem mimetics (TPP) and ester FP variants (O'Neill and Posner 2004). When haem models are used, since porphyrin alkylation is a favoured process, end-product distributions of products can be very different from when a free ferrous ion source is employed. Furthermore, solvent has been shown to have a profound effect on the products obtained in iron-mediated endoperoxide degradation. Thus all of these studies are truly only approximate models of the actual events within the malaria parasites (Posner and Meshnick 2001; Wu 2002). Future work is needed to correlate the results of biomimetic chemistry with the actual situation within the parasite.

5.2.2

Enzymes as Targets

As described earlier, erythrocytic malaria parasites degrade haemoglobin to acquire amino acids for protein synthesis. Falcipain 2 (FP-2; Dua et al. 2000) is a papain family cysteine protease that appears to act in concert with other enzymes including two aspartic proteases (Banerjee et al. 2003; Boss et al. 2003) to degrade haemoglobin. Incubation of erythrocytic parasites with inhibitors of FP-2 blocks haemoglobin degradation and parasite development. Pandey has demonstrated that, in purified digestive vacuoles from *P. yoelii*, cysteine protease activity can be inhibited by artemisinin in a similar manner to the potent cysteine protease inhibitor E-64 (Pandey et al. 1999). Inhibition of falcipain-mediated cleavage of the fluorogenic peptide substrate Z-Phe-Arg-AMC was also demonstrated in a continuous fluorometric assay, and surprisingly protease inhibition was increased in the presence of haem (surprising in the sense that strong arguments have been made that the haem-generated radical species cannot escape the porphyrin macrocycle and hit biological targets, post-reductive endoperoxide cleavage).

To fully validate falcipain 2 and 3 as targets for endoperoxide drugs, it is essential that these studies be expanded to human forms of the parasite. In addition, it would be of great interest to compare the efficiency of falcipain inhibition with the known antimalarial activities of a series of artemisinin analogues of varying potency.

Krishna and co-workers (Eckstein-Ludwig et al. 2003) have very recently provided compelling evidence that artemisinins act by inhibiting PfATPase6, the Sarco/Endoplasmic reticulum Ca^{2+} -ATPase (SERCA) orthologue of *P. falciparum*. When expressed in *Xenopus* oocytes, Ca^{2+} -ATPase activity of PfATP-

Pase6 is inhibited by artemisinin with similar potency to thapsigargin (another sesquiterpene lactone and highly specific SERCA inhibitor), but not by quinine or CQ. As predicted from this observation, thapsigargin antagonizes the parasitocidal activity of artemisinin. Desoxyartemisinin is ineffective as an antimalarial and was shown not to inhibit PfATPase6 activity. Chelation of iron by desferrioxamine abrogates the antiparasitic activity of artemisinins and correspondingly attenuates inhibition of PfATPase6. Single-cell imaging of living parasites with BODIPY-thapsigargin demonstrates cytosolic labelling that is competed by an excess of artemisinin. Furthermore, similar labelling is observed with a novel fluorescent artemisinin derivative. These studies support PfATPase6 as a target of artemisinins operating via an Fe^{2+} -dependent activation mechanism. This information may allow, for the first time, rational biological target-guided drug design efforts to be carried out.

5.3

Semi-synthetic and Synthetic Endoperoxide Analogues

The first generation C-10-acetal derivatives artemether (11c) and arteether (11d) both have a short half life as a consequence of cytochrome P450 catalysed transformation to dihydroartemisinin (DHA) (11b) which in turn is an efficient substrate for Phase II clearance through glucuronidation (Grace et al. 1998; Idowu et al. 1997; Maggs et al. 1997). In addition to metabolism, other first generation analogues such as artesunate are chemically unstable and hydrolyse rapidly to DHA in plasma [studies by Teja-Isavadharm indicate the half-life of artesunate ($t_{1/2}$) is as short as 0.41 ± 0.34 h in man following oral administration; Teja-Isavadharm et al. 2001; Barradell and Fitton 1995]. Based on these observations, medicinal chemists have made significant efforts to design more potent and stable analogues of the first-generation semi-synthetic derivatives. The metabolically more robust C-10 carba analogues 13a and C-10-aryl analogues of DHA 13b have been the focus of medicinal chemists for 10 years (Haynes 2001). Of note are the C-10 alkyl deoxo analogues prepared by Haynes et al. (2000), Posner et al. (1999), O'Neill et al. (1999, 2001b), Jung (Jung and Lee 1998) and Ziffer (Ma et al. 1999; Pu and Ziffer 1995) and the C-10 aryl or heteroaromatic derivatives 13c prepared by the groups of Haynes (Haynes et al. 2003a) and Posner (Posner et al. 2003; Fig. 5). Equally impressive are the C-14 modified analogues 13d prepared by the Avery (Vroman et al. 1999) and Jung groups (Jung et al. 2001).

Recently a C-10 carba analogue (TDR 40292) 13e has been compared with artemether. This compound cannot form DHA as a metabolite and contains a side chain that can be formulated as a water-soluble salt (Hindley et al. 2002). In addition, this compound has superior activity to artemether and artesunate, both in vitro and in vivo. From initial pharmacokinetic data,

13d has a higher volume of distribution than artemether and is considerably more orally bioavailable (16% versus 1.5% for artemether) (O'Neill et al. unpublished results). (For studies on the bioavailability of artemether in man see Silamut et al. 2003).

A particularly important factor in the design of any new peroxide analogue is the concern about potential neurotoxicity. Any analogue with a higher logP than artemether (3.3–3.5) is likely to cross the blood–brain barrier (Haynes, 2001). Haynes and co-workers have prepared new analogues with reduced neurotoxicity by applying the ADME paradigm for enhancing efficacy through increased drug absorption (coupled with a reduction in the ability of the new analogue to cross the blood–brain barrier). Artemisone (undergoing development by Bayer) is an analogue with much improved properties and represents the success of the ADME approach to drug design (http://www.mmv.org/pages/page_main.htm).

An alternative approach to preventing the formation of dihydroartemisinin by simple P450 metabolism is to replace the methyl function in artemether with an aryl function (O'Neill et al. 2001a). Phenoxy analogues of DHA can easily be prepared in a one-step synthesis from dihydroartemisinin. In addition to having superior in vivo activity to artesunate and artemether, analogues substituted with a *p*-fluoro (**13f**, R=*p*-F) or trifluoromethyl group (**13g**, R=*p*-CF₃) resist metabolism to DHA. In order to improve water solubility, a novel meta carboxylic acid phenoxy derivative (**13i**, R=*m*-CO₂H) has recently been prepared as a metabolically more robust alternative to artesunate and artemisinic acid.

With an ever increasing number of artemisinin analogues prepared by semi-syntheses and elegant total synthesis Avery (Avery et al. 1989; Vroman et al. 1999) has developed predictive 3-D quantitative SAR (CoMFA) analyses for the artemisinin class of antimalarial (Avery et al. 1994, 2002). This information coupled with the ADME approach described above should permit highly potent and orally bioavailable semi-synthetic analogues to be designed by a truly rational approach (Haynes, 2001).

The disadvantage of all of the semi-synthetic compounds is that their production requires **11a** as starting material. Artemisinin is extracted from the plant *Artemisia annua* in low yield (0.01–0.8% yield) (Klayman et al., 1984; Liu et al. 2003; Abdin et al., 2003). To circumvent this problem, a number of groups have produced totally synthetic peroxide analogues, some of which demonstrate remarkable antimalarial activity (Borstnik et al. 2002; Tang et al. 2004). These include the synthetic 1,2,4-trioxane, fenozan B0–7 (**14**) (Peters et al. 1993a, 1993b; Jefford et al. 1995, 2001) the dispiro tetraoxanes (**15**, **16**) (Vennerstrom et al. 2000) and the endoperoxide analogues such as arteflene (**17**) (Hofheinz et al. 1994) a synthetic analogue of the naturally occurring yingzhaozu A (**18**) (Zhou and Xu 1994). More recently, tetraoxane (**19**) with

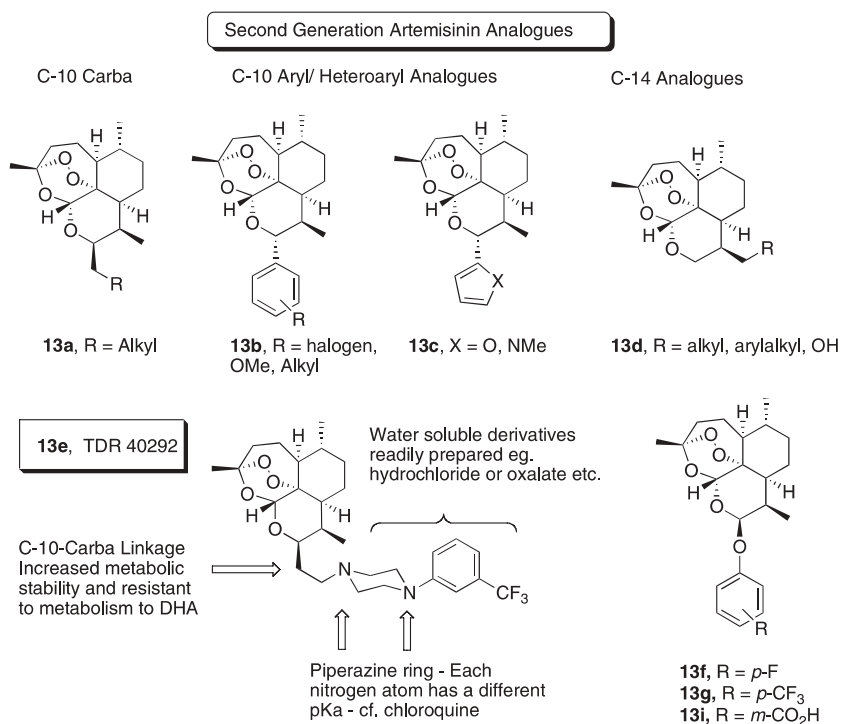


Fig. 11 Selected second-generation artemisinin derivatives

an IC_{50} as low as 3 nM has been discovered (artemisinin IC_{50} =10 nM) and analogues in this class have been shown to be effective when given orally in mice infected with *P. berghei* with no observable toxic side-effects (Fig. 11; Kim et al. 2003).

Other synthetic candidates worthy of mention include the C-3 aryl trioxanes (**20a**, R=F and **20b**, R=–COOH) and the endoperoxide analogue (**21**) (Posner et al. 1998; Korshin et al. 2002). These latter compounds have oral activity (ED_{50}) as low as 0.5 mg/kg in mice infected with *P. berghei* (Bachi et al. 2003).

The most significant recent discovery in this area is the discovery that easily synthesized ozonides (1,2,4-trioxolanes) substituted with an adamantane ring (**22a–c**) are not only chemically stable but are active against *P. falciparum* in the low nanomolar range. These compounds are orally active in mice and have a prolonged duration of action when compared with previously available synthetic and semi-synthetic derivatives. This research is supported by the MMV and a 2-year objective is to progress this project from preclinical

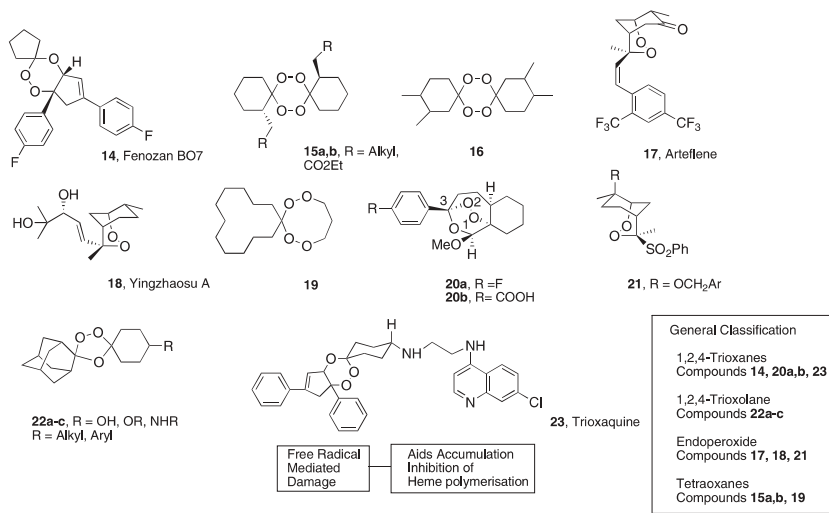


Fig. 12 Synthetic peroxide analogues

development to phase 1 clinical trials. Ranbaxy (an Indian pharmaceutical company) is in partnership with MMV to move the project forward. This project was the MMV project of the year in 2001, and excellent progress has been made since then. Water-soluble compounds that retain good oral activity in the *P. berghei* mouse model have been identified (Vennerstrom et al. 2004). These compounds have longer-lasting activity than current artemisinin derivatives, suggesting that treatment courses of 3 days or less are feasible (see: http://www.mmv.org/pages/page_main.htm).

The final class of analogue of note are the quinoline-peroxide hybrids known as trioxaquinines (23) (Fig. 12). These compounds have been designed in order to offset parasite resistance development and to aid parasitized erythrocyte penetration (Dechy-Cabaret et al. 2000, 2001). It is proposed that these hybrids may have the capacity to hit the parasite by two different mechanisms (namely, free radical mediated damage and interference with FP crystallization and detoxication).

6

Summary

In this chapter we have attempted to describe the exciting new advances in our knowledge of the mechanism of action and parasite resistance of two of the most important groups of semisynthetic antimalarials. Clearly we have

come a long way since the days of traditional herbal remedies but we are up against a sophisticated foe and we must not allow the drug resistant parasites to gain the upper hand. With that in mind we have presented some of the most exciting recent work on the design and synthesis of analogues of both quinoline and artemisinin/simplified cyclic peroxides. New design strategies encompassing hybrid drugs and identification of chemically and metabolically stable artemisinin derivatives have been explored. These efforts have produced promising candidates, some of which are undergoing preclinical evaluation at present.

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