Therapeutic Drugs for Targeting Chloroquine Resistance in Malaria

Vijay Sharma*

Mallinckrodt Institute of Radiology, Washington University Medical School, St. Louis, MO 63110, USA

Abstract: While the post-genomic era could lead into new targets for antimalarial drug development, herein few successful targets including medicinals involved in those processes are presented. Further, contribution of bioinorganic chemistry has also started to make its impact in the field of pharmaceuticals. Therefore, metal chelators, selected organometallics, and metalloantimalarials that would offer potential therapeutic drugs are also discussed. Finally, a brief summary on chloroquine-resistance mechanism(s) has been included.

Keywords: Therapeutic drugs, chloroquine, drug-resistance, metalloantimalarials, malaria.

INTRODUCTION

Malarial parasites emerging from the genus *Plasmodium* infect 300-500 million people and result in the death of 1-3 million children each year [1,2]. Recently, interdisciplinary efforts involving a consortium of several laboratories have resulted in genome sequencing of *Plasmodium falciparum* [3] and the mosquito vector *Anopheles gambiae* [4]. These genomic discoveries will provide enormous amount of information about the interaction of the parasite with its host and carrier, including the genes involved in parasite recognition by the human immune system. However, translating this information into effective remedies could be an immensely challenging task due to the resistance pathways devised by the parasite.

The most notorious of mosquitoes, the malaria vector *Anopheles gambiae* carries four species of parasites that infect humans, *Plasmodium falciparum*, *Plasmodium vivax*, *Plasmodium malariae*, and *Plasmodium Ovale*; the most deadly of which is *P. falciparum* which accounts for approximately 70% of cases in sub-Sahara Africa and 50% of cases in Asia and Latin America.

LIFE CYCLE OF PARASITE

The malarial parasite leads an extremely complicated life. While existing inside the human host, it primarily resides within liver cells and red blood cells, while its life within mosquitoes involves infection of the insect's gut and salivary glands. Parasites undergo several transformations based upon morphological stages that can be categorized into merozoites, trophozoites as well as gametocytes within humans and zygote, ookinete including sporozoites in mosquitoes. Typically, while feeding on a human host, parasite-infected mosquitoes inject sporozoites. These sporozoites travel to the liver, mature through several stages, and finally produce merozoites that invade and multiply via the trophozoite stage in red blood cells. Eventually 10% or more (*P. falciparum*), and 1% or less (for other species) of all red blood cells become infected. The merozoites within these infected red blood cells develop into gametocytes. Further, when another mosquito bites an infected human, it takes up blood possessing gametocytes that produce reproductive cells (gametes) which in turn fuse within insect's gut to form zygotes. Thereafter, zygotes change into the ookinete that cross the wall of the gut and form a sporozoite filled oocyst. When an oocyst bursts, the sporozoites move to the mosquito's salivary glands and the process begins again [5].

*Address correspondence to this author at the Mallinckrodt Institute of Radiology, Washington University Medical School , 510 S. Kingshighway Blvd., Box 8225, St. Louis, MO 63110, USA; Tel: 314-362-9358; Fax: 314-362-0152; E-mail: sharmav@mir.wustl.edu

TARGETS FOR DRUG DEVELOPMENT

The efficacy, affordability, oral bioavailability, and low cytotoxicity profiles of chloroquine have contributed to reducing mortality and morbidity rates of malaria across America, Africa, and Asia. In spite of easy access and distribution, chloroquine-resistance strains have slowly emerged in the underdeveloped regions of world. Thus, an urgent need exists for an inexpensive antimalarial drug that mimics the advantages and potency profiles of chloroquine (CQ), yet is able to overcome resistance mechanism(s). The discovery of new antimalarial therapeutics poses further challenges as the potential drug must satisfy two vital criteria: a) the targeted enzyme, receptor or process must be absent in the host or significantly different between the parasite and host to ensure target specificity; and b) the targeted enzyme or process must be critical to parasitic growth and survival. In addition, the mechanism of action for antimalarial agents such as CQ that target intracellular hemozoin necessitates transport across four bilayers: the erythrocyte plasma membrane; the parasitophorous vacuolar membrane; the parasite plasma membrane; and finally the digestive vacuole membrane.

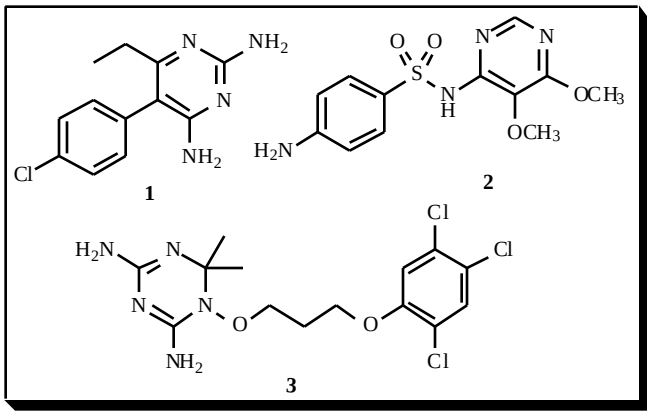
While the post-genomic era promises new information for identifying novel targets for drug development, a few of the successful targets in traditional antimalarial drug development are presented here:

A. Dihydrofolate Reductase

Dihydrofolate reductase (DHFR), a key enzyme in the folate metabolic pathway, is an important target for antimicrobial drugs. Its inhibition depletes the cellular pool of tetrahydrofolate, a cofactor that is essential for both DNA and protein synthesis. Thus, specific inhibitors have been designed for both prokaryotic and eukaryotic pathogens. However, selectivity for pathogenic DHFR over host enzyme

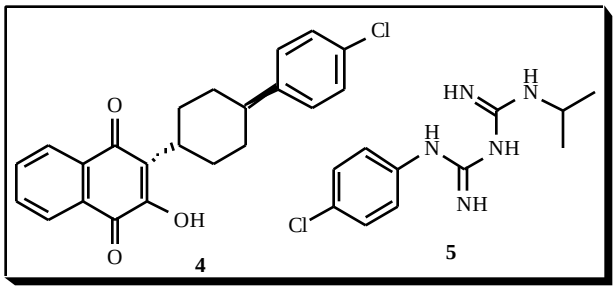
1389-5575/05 $50.00+.00

is an essential feature in the development of DHFR inhibitors. Pyrimethamine **1** is a specific inhibitor of DHFR from *P. falciparum*, and its combination with sulfadoxine **2** in a ratio of 1:20 forms a new combination therapeutic drug, Fansidar. However, resistance to pyrimethamine in *P. falciparum* has been attributed to the mutations in the *dhfr* gene. Interestingly, a triazine derivatized compound, WR99210 **3**, a metabolite of a potential biguanide prodrug, has been shown to be active against pyrimethamine-resistant mutants of *P. falciparum* and *P. vivax* [6,7]. In addition, selectivity for the pathogenic DHFR over host has been an interesting feature of this drug. It has been postulated that binding of **3** to the human enzyme releases DHFR mRNA leading to new protein synthesis and circumvention of its effect on the host. Furthermore *Plasmodium* DHFR is a bifunctional enzyme consisting of DHFR and thymidylate synthase (TS) in which the mRNA transcript does not bind to the catalytic domain of DHFR, but rather to a linker region between DHFR and TS. Consequently, binding of drug does not cause liberation of mRNA, thereby rendering the parasite unable to synthesize new enzyme required for its survival [8].



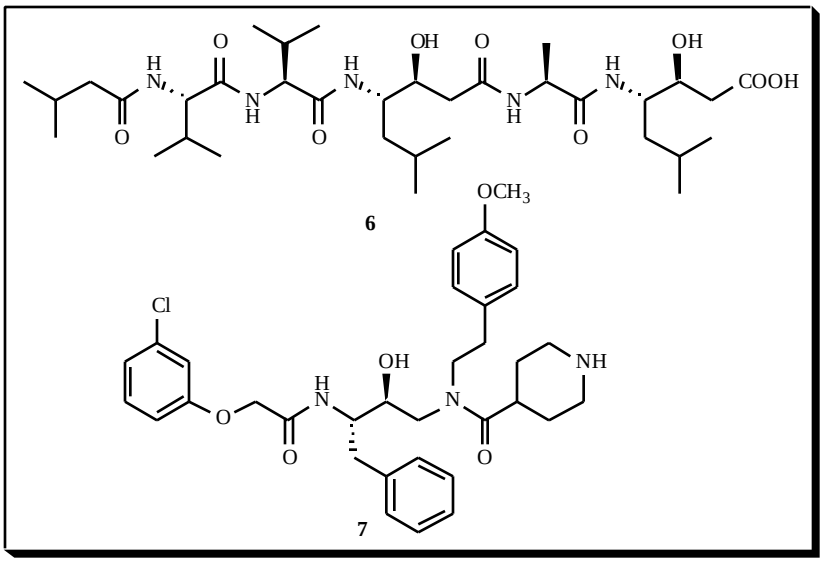
B. Electron Transport (Cytochrome b/cl Complex, or Cytochrome c Reductase)

Drugs have been designed to selectively target mitochondrial functions of malarial parasites compared to mammalian hosts [9]. The hydroxy-quinones have been explored as potential antimalarials due to their ability to influence respiratory and carbohydrate metabolism of malarial parasites [10], however their toxicity and metabolic instability contributed to their demise. Recently, a derivative of hydroxy-naphthoquinone, namely atovaquone, [2-{trans-4-(4'-chlorophenyl)cyclohexyl}-3-hydroxy-1,4-naphthaquinone]**4,** has been shown to inhibit the cytochrome bc_1 (CYT bc_1) complex of the electron transport chain of the malarial parasites [11]. Furthermore, studies of mitochondrial functions have indicated that this drug collapsed mitochondrial membrane potential and inhibited respiration of the parasites selectively over their mammalian host [12]. In addition, while evaluating a combined therapeutic effect of atovaquone **4** and proguanil **5**, Srivastava and Vaidya have shown that **5** enhances the collapse of membrane potential without impacting electron transport inhibition due to **4** [13], indicating success of combined drug in the geographic regions that have encountered resistance to proguanil **5**.



C. Hemoglobin Catabolism

Malarial parasites require nutrients (amino acids) for their growth and survival. Therefore, the parasite has devised a unique pathway to obtain these essentials through catabolism of host hemoglobin (Fig. **1**), an oxygen transport protein [14]. Consequently, approximately 80% of the host's infected erythrocyte hemoglobin is degraded during the intraerythrocytic stages of the parasitic life-cycle. This hemoglobin is endocytosed from erythrocyte cytosol, and trafficked to an acidic lysosomal compartment known as the digestive vacuole (pH 5.2-5.5). Various *Plasmodium*



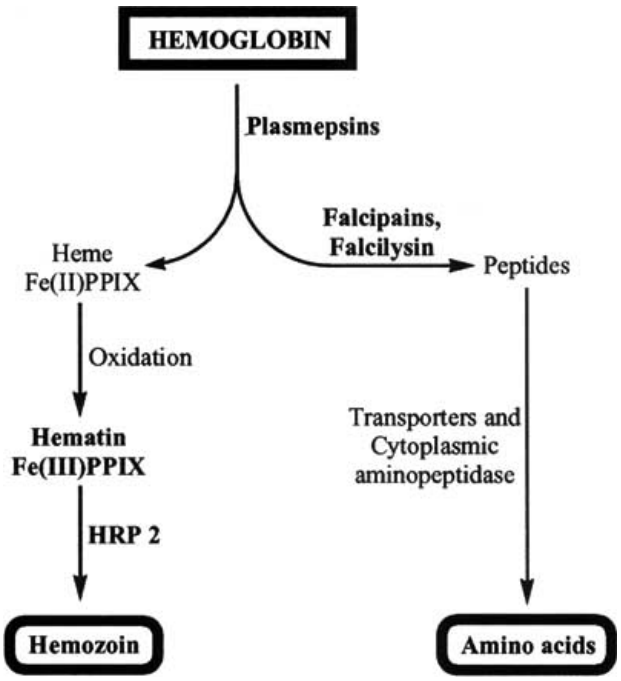


Fig. (1). Catabolic pathway for degradation of hemoglobin in the digestive vacuole of *P. falciparum.*

proteases have been identified that mediated vacuolar degradation processes. Aspartic proteases are common in eukaryotes, and these enzymes, known as plasmepsins in the malarial parasite *Plasmodium falciparum*, provide an excellent target for antimalarial drug development [15,16]. In this family of enzymes, a water molecule serves as a nucleophile to attack the scissile peptide bond in a target substrate. This water molecule is held in position and activated by two aspartic acid residues. Goldberg and coworkers [17] have shown that enzymes such as plasmepsins (I & II) initiate this degradation process by cleaving hemoglobin between residues α33Phe and α34Leu in a highly conserved hinge region of the domain that holds the hemoglobin tetramer together, when bound to oxygen. Cleavage of this hinge region subsequently induces proteins that promote proteolysis. Pepstatin **6**, a potent inhibitor of most aspartic proteases, has been shown to inhibit degradation of hemoglobin through analysis of digestive vacuole extracts. Using recombinant plasmepsin II, the inhibition constant for pepstatin A was found to be 0.006 nM [18]. In addition, both plasmepsin II and cathepsin D possess 116 common amino acids (homology 35%). Therefore, various cathepsin D inhibitors have also been evaluated for cross-inhibition. [18] Recently, Ellman and co-investigators have identified a series of potent plasmepsin II inhibitors **7** through combinatorial chemistry and structure-based rational drug design [19].

Further degradation of hemoglobin is obtained by conversion of large protein fragments into small peptides and the process has been reportedly mediated by falcipains [20], cysteine proteases, that resembles in substrate specificity to cathepsin L [21]. Thus, various chalcone and acyl hydrazide derivatives that target falcipains have been shown to inhibit cathepsin L [22]. Furthermore, various quinolinyl chalcones have been obtained, and found to possess moderate efficacy against falcipains [23]. Goldberg and coworkers have suggested the existence of a metalloprotease, falcilysin, in the digestive vacuole that further act on small peptides in the hemoglobin degradation pathway [24,25]. Falcilysin has

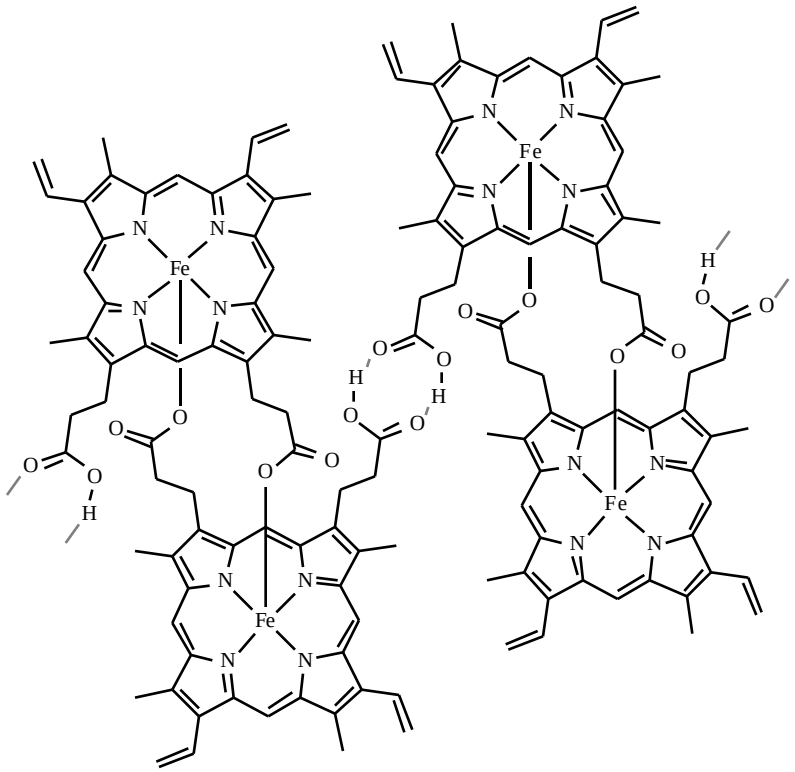
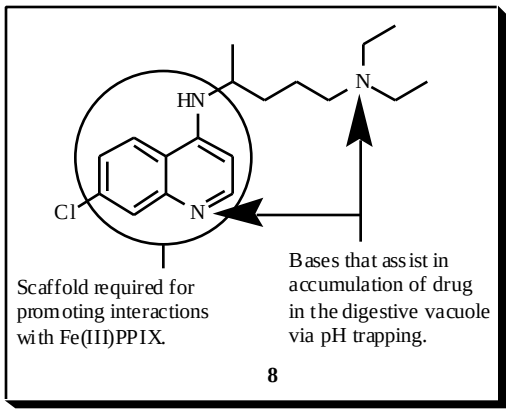


Fig. (2). Structure of hemozoin based upon current X-ray model.

been postulated to function downstream of the aspartic proteases, plasmepsin I and II, and the cysteine proteases, falcipains, in the hemoglobin proteolytic pathway. However, it is unable to cleave hemoglobin or denatured globin but readily destroys peptide fragments of hemoglobin [24]. Finally, these small peptides are transported out of the digestive vacuole into the cytoplasm. This process is mediated by transporters located on the vacuolar membrane. This aspect will be discussed in more detail in the section on the chloroquine resistance mechanism(s).

D. HEMOZOIN FORMATION

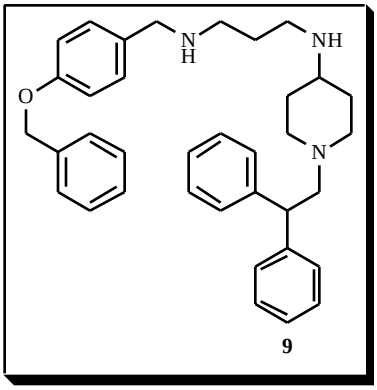
While catabolism of hemoglobin **(**Fig. **1)** produces needed amino acids for parasitic growth, it also produces toxic, free heme in the digestive vacuole wherein the central core iron(II) of heme is oxidized to iron(III) (ferriprotoporphyrin IX; Fe(III)PPIX; FPIX). Overproduction of heme can disrupt metabolic functions of membrane bilayers, inhibit enzymes and generate oxidative free radicals [26,27]. To balance the metabolic needs for amino acids versus the toxic effects of heme, the parasite has evolved a unique detoxification process that involves formation of a crystalline, insoluble, black polymer, commonly known as hemozoin. The structure of this bio-crystal was found to consist of dimers of the five coordinate FPIX linked by reciprocating monodentate carboxylate linkages from one of FPIX's propionate functionalities [28]. These dimers are linked via an extensive network of hydrogen bonds contributed by the second propionic acid group of FPIX (Fig. **2**). While determination of the hemozoin structure has resolved scientific debate regarding the chemical nature of this target, the process of its formation *in vitro* and *in vivo* continues to be an area of intense investigation [29].



8

Despite debate over mechanism(s) of hemozoin formation [29], it has been considered to be an attractive target for generation of new antimalarial drugs due to following reasons: a) targeting of parasites involves stages that are causing clinical pathologies; b) humans are believed to detoxify free heme via the heme oxygenase/biliverdin reductase pathway. Therefore, a given drug that putatively interferes with heme biocrystallization in the parasite would not impact the heme detoxification process in humans; and finally c) inhibition of this process would lead to accumulation of high concentration of toxic heme inside the parasite that would be detrimental to its existence. Thus, inhibition of this vital biocystallization process [30] is postulated to be the mechanism of action of antimalarial quinolines such as chloroquine (CQ) **8** [14,31].

In addition, histidine-rich proteins (HRP) have also been shown to play a pivotal role in hemozoin nucleation inside digestive vacuoles of *P. falciparum*. Among HRP's, HRP II possesses a high content of histidine (34%), alanine (37%) and aspartic acid (10%) with contiguous repeats amino acid sequences of the tripeptide (AHH) and pentapeptides (AHHAA) [32]. Due to its high content of histidine, HRP II has been postulated to bind metals. Specifically, its affinity for Zn^{2+} ion has been employed as a tool to isolate extracellular protein [33]. Sullivan *et. al.* have demonstrated that hemozoin formation may also be mediated by two histidine-rich proteins, HRP II (30 kDa) and HRP III (27 kDa) [34]. Exploiting the rich reservoir of histidine in its sequence, HRP II also has been shown to bind heme. Therefore, drugs that inhibit heme binding to HRP II or inhibit polymerization of heme bound HRP II, induce toxicity to parasite. While developing a microtiter plate assay for evaluating inhibition of heme binding to HRP II to screen combinatorial libraries, a lead compound, N-[1-(2,2-Diphenyl-ethyl)-piperidin-4-yl]-N'-(benzyl-4-benzyloxy)-propane-1,3-diamine **9** has been shown to interfere with HRP II binding to heme. In addition, **9** was found to be active against both chloroquine-sensitive and –resistant strains [35,36]. Results have indicated a linear relationship between parasitic death and inhibition of HRP II binding to heme, confirming HRP II as another attractive target for antimalarial drug development.



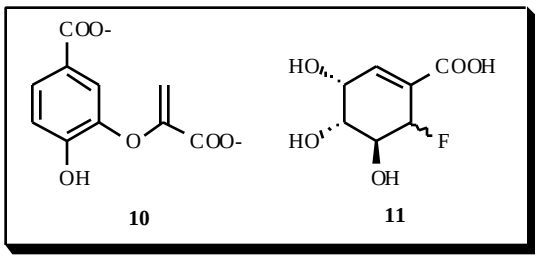
9

E. Shikimate Pathway

The Shikimate pathway, also known as the "aromatic biosynthetic pathway", involves generation of a common aromatic precursor, chorismate **10,** from erythrose-4-phosphate and phosphoenolpyruvate in various enzymatic steps. Despite debate on its localization, the presence of this pathway in *Plasmodium*, and its absence in mammals, makes it another target for antimalarial drug development [37]. Various fluorinated analogues of shikimate **11** have been found to inhibit the growth of *Plasmodium* culture at sub-micromolar concentrations [38].

Finally, the apicoplast has also been identified as an increasingly important target in the development of antimalarial therapeutics [39]. It contains a range of metabolic pathways and housekeeping processes that are radically different to those of host therefore present ideal strategies for drug therapy [40]. Thus, inhibitors of the nonmevalonate

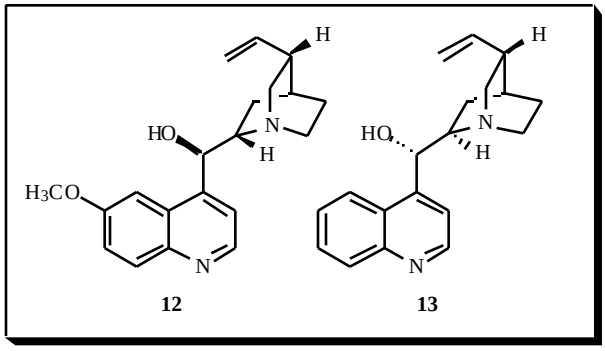
pathway of isoprenoid biosynthesis have been found to possess antimalarial efficacy [39].



10 **11**

ANTIMALARIAL DRUGS

Historically, the first quinoline based antimalarial drugs were extracted from the cinchona tree, named after the countless Chinchon who, according to legend, were cured of malaria in 1630 by a powder made from its bark. The active compounds (quinine **12** and cinchonine **13**) were isolated from a crude mixture of crystalline alkaloids. Woodward *et. al.* reported the first synthetic pathway for quinine [41,42]. During World War II, due to shortage in supply of quinine, synthetic alternative therapeutic drugs such as chloroquine **8** and pyrimethamine **1** replaced quinine **12** for prophylaxis and routine treatment. For decades, people in underdeveloped regions of the world have relied upon chloroquine for treatment of malaria due to its easy access, wide distribution, and minimal side effects. However, emergence of chloroquine-resistant strains has led to the exploration of alternative therapeutic drugs.



12 **13**

A. Quinoline Based Drugs

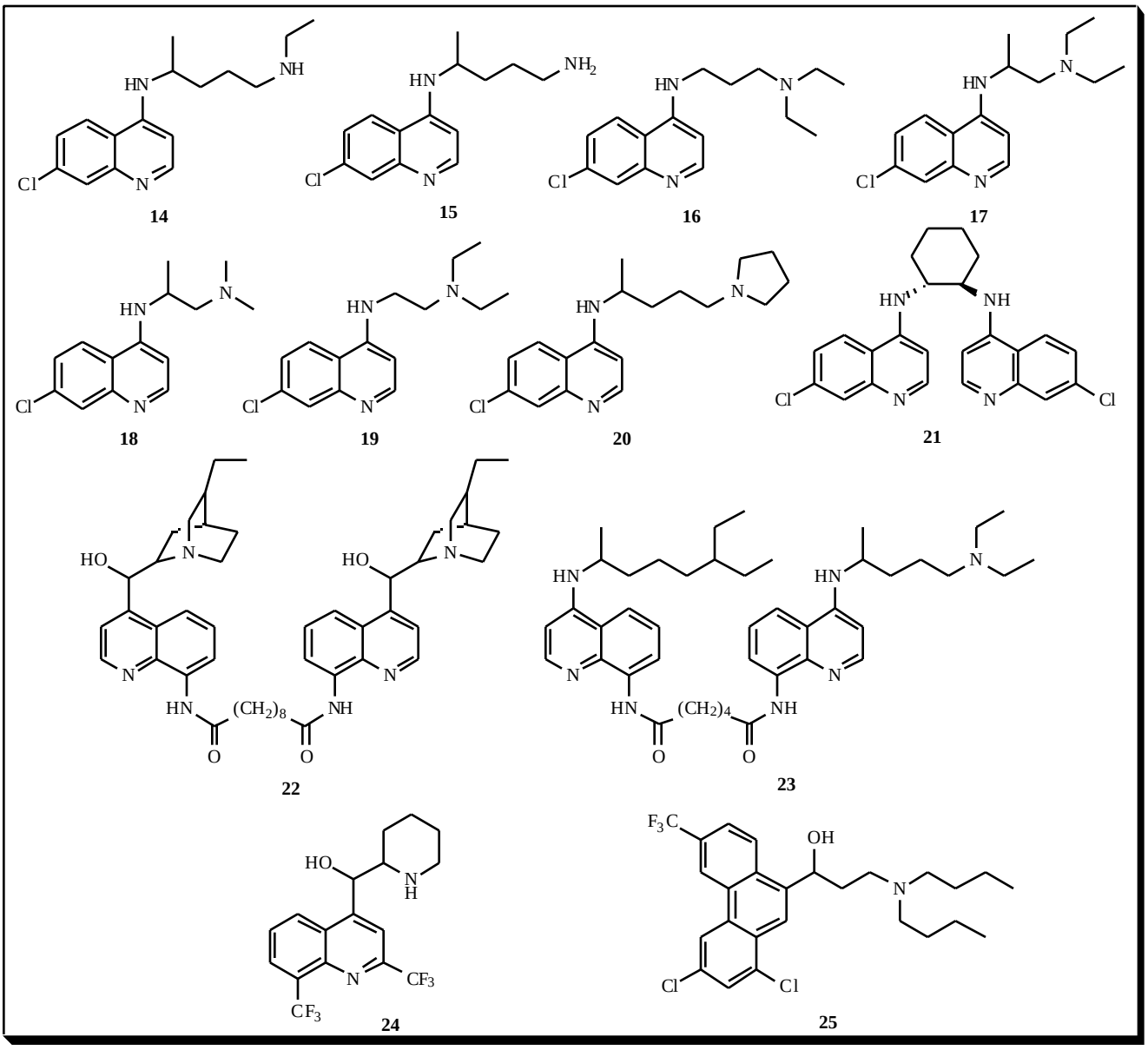
Chloroquine (CQ) **8**, the classic hemozoin-targeted agent, is a moderately hydrophobic base possessing titratable protons that confer net positive charge in the acidic environments of digestive vacuole. Thus, CQ is thought to diffuse in its non-protonated form across the vacuolar membrane, and be trapped in the acidic compartment of the digestive vacuole [43,44]. Once in the vacuole, chloroquine prevents sequestration of toxic heme into hemozoin by binding heme. The ability of CQ-like drugs to act as inhibitors of heme aggregation may be dependent upon two factors: a) formation of drug-heme complex; b) interaction of the drug-heme complex with the heme polymer. Furthermore, hemozoin from both CQ-sensitive and -resistant strains has been found to have similar affinity for the CQ-heme complex, thereby suggesting failure of CQ to access its target site as a major factor in drug-resistant strains [45].

Although hundreds of quinoline-based antimalarials [14,46,47] have been investigated, the rational drug design involving structure-activity relationships of chloroquine primarily focussed around three major variations: a) modification of side-chain substituted on the 4-position of the quinoline ring (monodesethyl chloroquine (DECQ) **14**; bidesethylchloroquine (diDECQ) **15**; Ro-47-0543 **16**; Ro-48-0346 **17**; Ro-47-9396 **18**; Ro-41-3118 **19**, chloroquine-pyrollidinyl **20**); b) retention of an electronegative substituent on the 7-position of the quinoline ring and c) derivatized quinolines attached through dialkylamines WR-268668 **21** or 4-substituted bis-quinolin-8-yl amides **22, 23** [48]. In addition, 4-quinolinemethanols, such as mefloquine **24**, and 9-phenanthrenemethanols, such as halofantrine **25** have shown activity against CQ-resistant strains. Despite activity against resistant strains, utility of halofantrine has been restricted due to its cardiotoxicity. Recently, using isothermal titration calorimetry, Vippagunta *et al.* have shown that derivatized 4-amino-quinolines interact with hematin-μ-oxo-dimers resulting in a cofacial π-π sandwich type complex [49]. However, variation in the position of electronegative atom on the quinoline or its replacement with a donor substituent resulted in the loss of antimalarial activity indicating that the chlorine substituent at 7-position is a critical structural factor in determining antimalarial activity of 4-aminoquinolines [49].

In recent years, cation-π type interactions have been recognized as an important noncovalent binding force [50], and have been postulated to be a dominant force in biological environments with a number of protein systems [51,52]. Therefore, based upon differences in the cation-π type non-covalent interactions between aromatic rings (chloroquine **8** and mefloquine **24**) and metal ions, it has been demonstrated that π electrons of chloroquine ring are better suitable for various interactions exemplified as π-cation, π-π, π-charge, and π-dipole interactions compared with mefloquine that possesses highly electronegative substituents such as trifluoromethyls. Resultantly, chloroquine does bind to hematin due to favorable electronic profiles and this type of chemical interaction is absent in the case of mefloquine, consistent with its non-favorable electronic profiles [53]. In addition, Leed *et. al.* evaluated interactions of antimalarial drugs with FPIX in solution through high resolution NMR experiments [54]. The results indicated that aliphatic chain of chloroquine stabilizes the FPIX-CQ complex. Furthermore, lengthening of the chain more than three carbons would likely force the end of the CQ-side chain outside the rim of FPIX; conversely, shortening this side chain would negatively impact the van der Waals forces, indicating a critical role of aliphatic chain of CQ in its interactions with FPIX.

B. Artemisinin Based Drugs

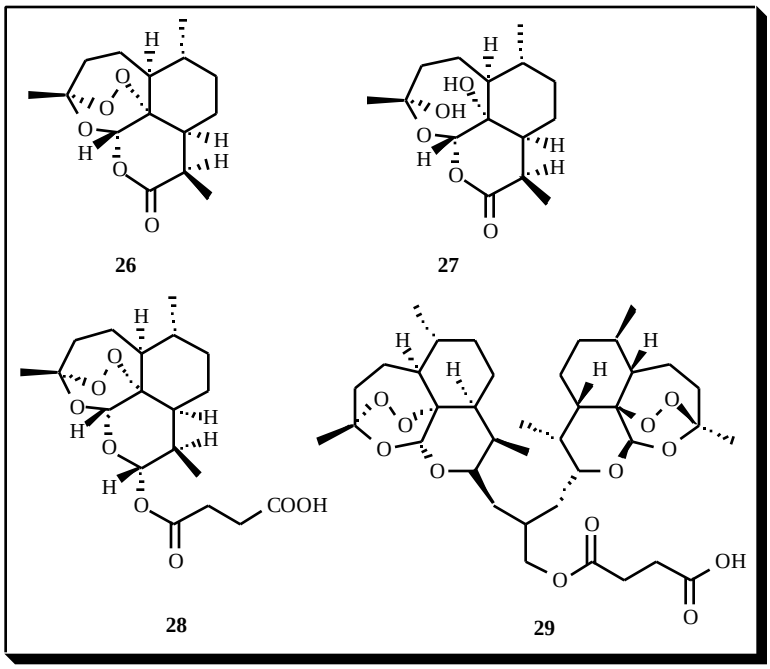
The emergence of resistant strains made it mandatory for scientists to broaden their efforts to look into alternative effective antimalarial drugs. Thus, a significant contribution in the field of malarial chemotherapy has been identification of the active component, artemisinin **26,** in the plant extracts of *Artemisia annua*, [55] commonly known as qinghaosu for treatment of malaria in China for over a thousand years. Despite promising biological activity, difficulties in the formulation of artemisinin lead to discovery of its water-



soluble counterparts, the derivatives of dihydroartemisinin **27**, and artesunate **28**. The compounds are believed to be activated by the iron-rich environment inside the parasite, wherein the trioxane gets chemically reduced by iron(II) resulting in disruption of the peroxidic oxygen-oxygen bond and production of an oxygen centered free radical. Generation of such a reactive chemical entity produces several chemical transformations. Among these chemical processes, *Posner et. al*. have identified the 1,5-hydrogen shift (Fig. **3**) as an important chemical migration that generates a stable carbon-centered free radical associated with antimalarial activity [56,57]. Finally, carbon radical fragments release a stable organic vinylic ether and a reactive iron-oxo intermediate [58] that induces toxicity in the parasite either by oxygenating and disrupting function of various biomolecules or by generation of epoxide by epoxidation of the vinylic ether. Compared with simple peroxides that are cleaved in the presence of reducing agents, the peroxide linkage of these trioxanes has been shown to be chemically inert, thus Posner *et al*. have modified the linker to create extremely potent, water-soluble carboxylic acid derivatives of artemisinin **29** [59,60].

C. Tetraoxanes

Due to existence of a correlation between the presence of peroxide functional group and antimalarial activity [61], a novel class of tetraoxanes has been evaluated for its antimalarial properties [62]. Among synthetic peroxides, dispiro-1,2,4,5-tetraoxanes exemplified as WR 148999 and related analogs **30** have been found to possess antimalarial activity comparable to **26** [63,64]. In addition, a steroid derivative appended with a critical pharmacophore, tetraoxacyclohexane **31** has been shown to be remarkably potent against both D6 (CQ-sensitive) and W2 (CQ-resistant) clones [65].

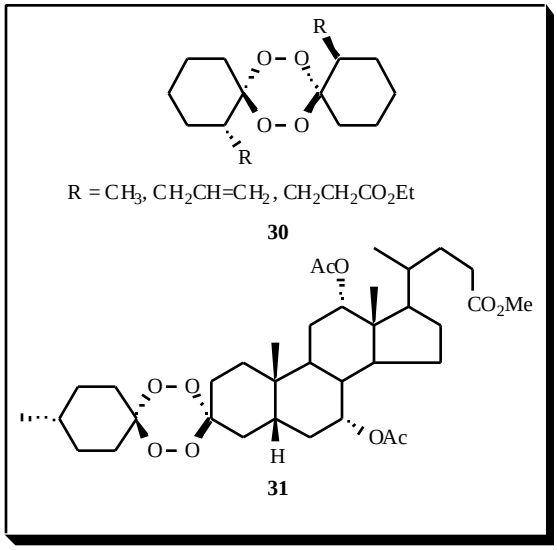


METAL COMPLEXES IN MALARIA

In the following three sections, review summarizes the classes of bioinorganic compounds that have shown promising efficacy as antimalarials, including metal chelators used as free ligands to bind metabolically active metals such as iron(III) as well as recently reported organometallic compounds, metal complexes of existing antimalarial drugs, and metalloantimalarials.

A. Metal Chelators in the Treatment of Malaria

Given the importance of iron metabolites in malarial physiology and toxicity, various metal chelators have been used as antimalarials [66,67]. The strategy is based upon the principles that, a) all microorganisms need iron for their growth and replication, b) microorganisms secrete siderophores, small molecular weight, water-soluble molecules that bind extracellular iron(III) with high affinity, the resultant metal complexes binding to selective surface receptors for internalization through a multi-step process, and c) iron deprivation impacts preferentially parasite growth relative to mammalian cells [43]. Therefore, an effective antimalarial chelator would be expected to possess characteristics exemplified as: a) ability to cross lipid membrane bilayers; b) possess high affinity for iron(III) over iron(II); c) offer six coordination sites for inherent stability of metal complexes (while pentadentate or quadridentate ligands may result in generation of one or two free coordination sites, the tridentate or bidentate ligands would result in the formation of 2:1 or 3:1 stoichiometric complexes leading to partial dissociation reactions. In either case, a net result would be availability of free coordination sites for participation in reactions to induce toxicity).



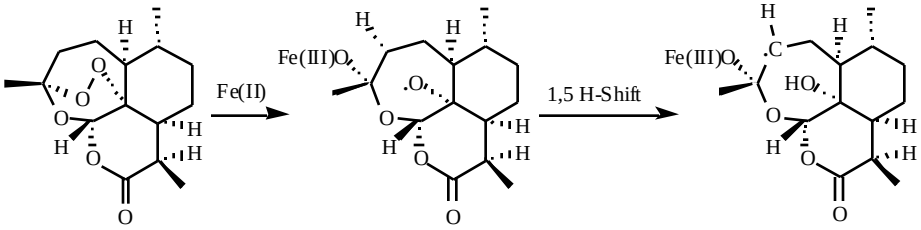
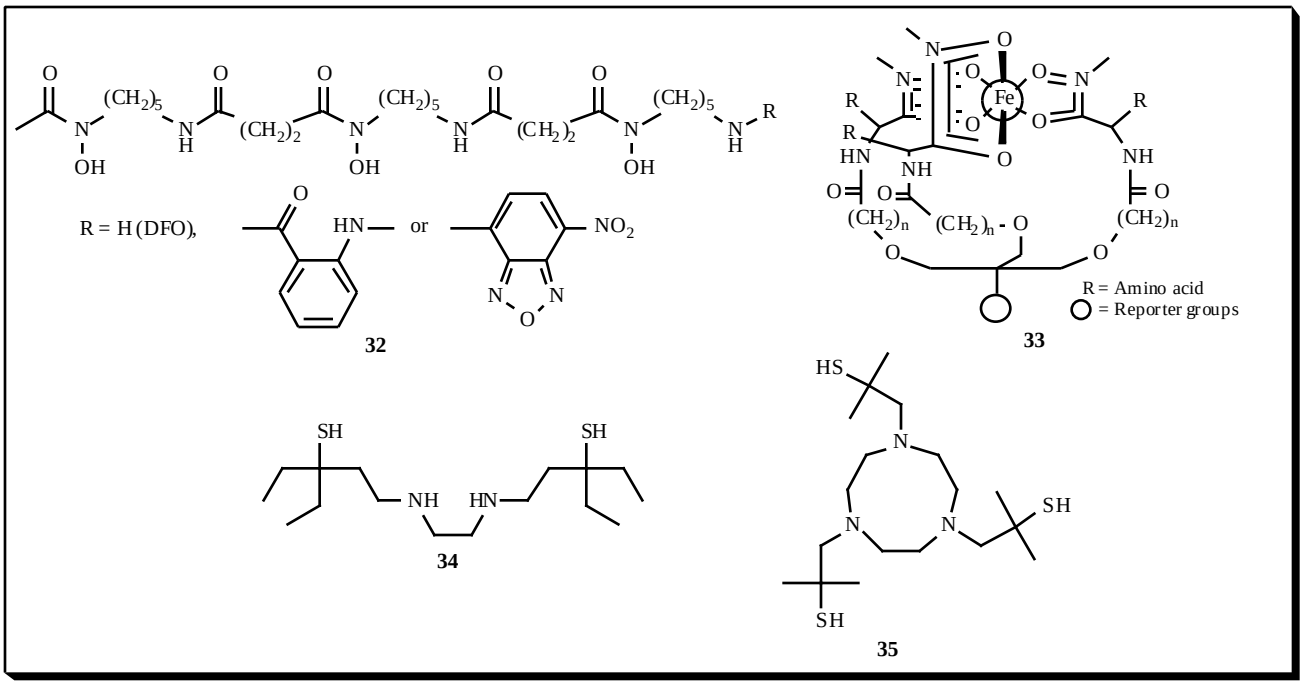


Fig. (3). 1,5-hydrogen shift as an important chemical migration to generate a stable carbon-centered free radical for antimalarial activity.



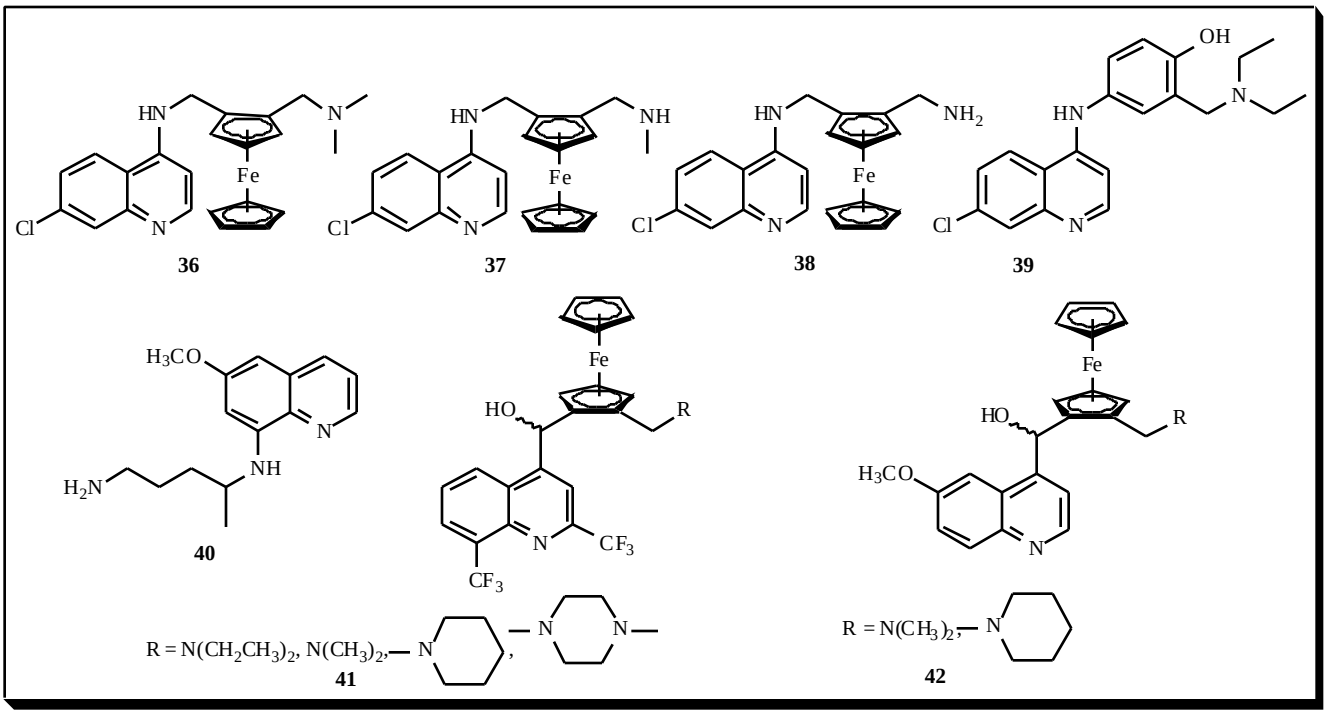
Thus, iron-chelators such as desferrioxamine (DFO) **32,** its derivatives, [66,68-71] other iron-chelating drugs such as polyanioinic amines [72], 3-hydroxypyridin-4-ones [73], and reversed siderophores **33** with tripodal design for potential incorporation of the fluorescent or radioactive markers as reporter groups [74,75] have been evaluated as antimalarials. The antimalarial effect of these chelators is attributed to their interaction with a labile iron pool within parasitized erythrocytes [70], and possible chelation of iron(III) in association with hemozoin [76], thereby depriving the parasite of a necessary trace metal. Previously synthesized for imaging applications [77,78] and known to bind iron with high affinity, aminethiol multidentate chelators such as 1,2-bis(3-ethyl-3-mercapto-pentylamino)-ethane (BAT) **34** and *N',N',N'*-tris(2-methyl-2-mercaptopropyl)-1,4,7-triazacyclononane (TAT) **35** were evaluated for their efficacy as antimalarials [79]. These compounds inhibited parasitic growth as evaluated through ^{3}H-hypoxanthine incorporation with IC_{50} values of 7.6 and 3.3 μM, respectively, and found to be 5-10 times more potent than desferrioxamine [79]. Based upon earlier data demonstrating the low stability constants for zinc(II) complexes of DFO versus iron(III) complexes of DFO [80], a zinc(II)-DFO complex was synthesized recently as a potential antimalarial compound [81]. Thought to possess improved membrane permeability properties, the results suggested that zinc(II) complexes were engaged in transmetallation reactions with iron(III) within intracellular compartments; the strategy provided improved potency in inhibiting the growth of parasites compared with DFO alone.

However, it could be argued that metal chelators would lack selective targeting properties, and therefore, undesired side effects may appear in the host. Thus, strategic use offree ligands for antimalarial applications may result in formation of complexes with other essential metal cations [unless binding constants are extremely favorable for iron(III)] in the extracellular and intracellular compartments of the host, thereby leading to unanticipated biological effects.

B. Organometallic Compounds as Antimalarials

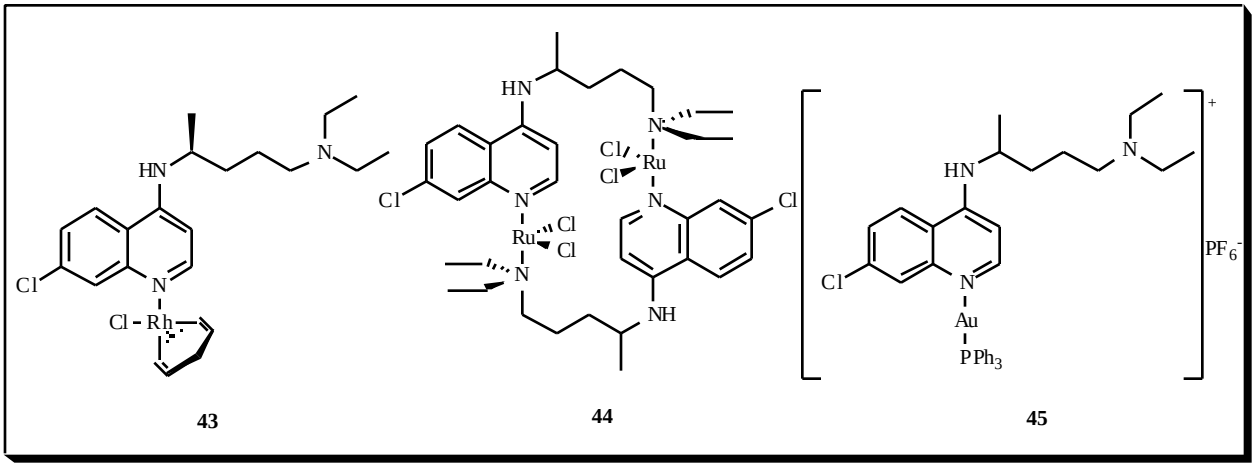
Encouraged by the success of cis-platin and several other platinum compounds as antitumor agents as well as gadolinium complexes as contrast agents for magnetic resonance imaging [82], bioinorganic chemists have begun to evaluate the utility of intact metal complexes as potential antimalarials. Herein, we report recent efforts to design metal complexes for antimalarial applications.

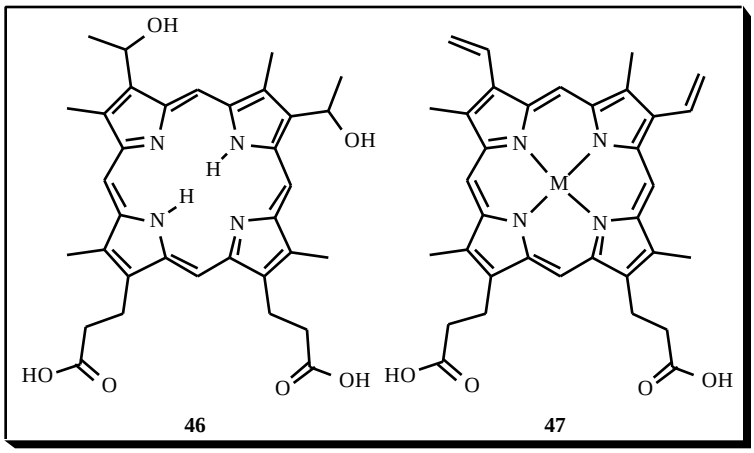
Organometallic compounds like ferrocene (dicyclopentadienyl-iron(II)) are stable and non-toxic thus, providing compatibility for biomedical applications. These eighteen electron systems (ten from rings and eight from iron in the zero oxidation state) are amenable to a variety of aromatic substitutions [83-85]. Recently, the carbon chains of chloroquine were substituted with a hydrophobic ferrocenyl group, while maintaining the position of two exocyclic nitrogens. Using this synthetic strategy [86], a ferrocene-chloroquine analogue, 7-chloro-4-{([2-{(*N,N*-dimethylamino)methyl}ferrocenyl]-methyl)-amino}quinoline (ferrochloroquine, FCQ) **36** was obtained. Compared with chloroquine **8**, **36** was 22 times more potent against drug-resistant strains of *P. falciparum*. Analysis of structure-activity relationships demonstrated that ferrocene was required to be covalently bound to chloroquine to antagonize drug resistance of the parasites. Thus, while ferrocene alone did not show any efficacy, ferrocene enhanced the potency of chloroquine when enclosed within the molecule [87]. It has been shown that chloroquine undergoes metabolism in the liver through a process mediated by cytochrome P-450 to produce metabolites monodese-thylchloroquine (DECQ) **14** and didesethylchloroquine (diDECQ) **15** and metabolites have shown less efficacy *in vitro* compared with parental chloroquine. Based upon this observation, it has been postulated that **36** may likely undergo similar metabolism [88] to produce desmethylferrochloroquine (DMFCQ) **37** and didesmethylferrochloroquine (diDMFCQ) **38**. Compared with chloroquine, Biot *et al*. have shown that these



metabolites are more efficacious against CQ-sensitive (HB3) and –resistant (Dd2) strains [88]. In addition, recent results have indicated superior potency of ferrochloroquine **36** compared with chloroquine **8**, quinine **12**, mefloquine **24**, amodiaquine **39** and primaquine **40** against 103 Gabonese isolates of *P. falciparum* [89]. Employing the same strategy of incorporation of ferrocene into existing drugs, analogues of mefloquine **41** and quinine **42** have been obtained [90]. While the mefloquine analogue was obtained through coupling of an aminomethyl substituted ferrocene carboxaldehyde with a lithioquinoline compound, the quinine analogue was prepared through inverse reaction of a lithioaminomethyl ferrocene with quinoline carboxaldehyde. Compared with native mefloquine or quinine, these compounds have shown decreased efficacy in the CQ-sensitive (HB3) and –resistant (Dd2) strains.

Exploiting the potent antimalarial activity of chloroquine against many malarial strains, direct incorporation of CQ into metal complexes or incorporation of CQ into the organic scaffolds of metal complexes has been explored. For example, treatment of bis(cyclooctadiene)rhodium(I) chloride [91] with 3 equivalents of chloroquine under mild conditions provided air-stable yellow micro-crystals of a metal complex **43**. Alternatively, interaction of ruthenium(III) chloride hydrate with 5 equivalents of CQ in the presence of a reducing agent provided another metal complex **44** [92]. Growth inhibition studies indicated that rhodium complex **43** was equipotent to chloroquine diphosphate (CQDP), whereas ruthenium complex **44** was 4 times more potent than chloroquine diphosphate against strains of *P. berghei*. However, the ruthenium complex was about four times more potent than CQDP in FCB1 strains and two times more potent than CQDP in drug-resistant FCB2 strains of *P. falciparum* [92]. In further efforts to obtain metal complexes of greater efficacy, the investigators isolated a gold complex of chloroquine **45** through a reaction of (triphenylphosphine)gold(I) chloride [93] in the presence of potassium hexafluorophosphate with 2 equivalents of CQ under reflux conditions [94]. The gold complex **45** was a more potent





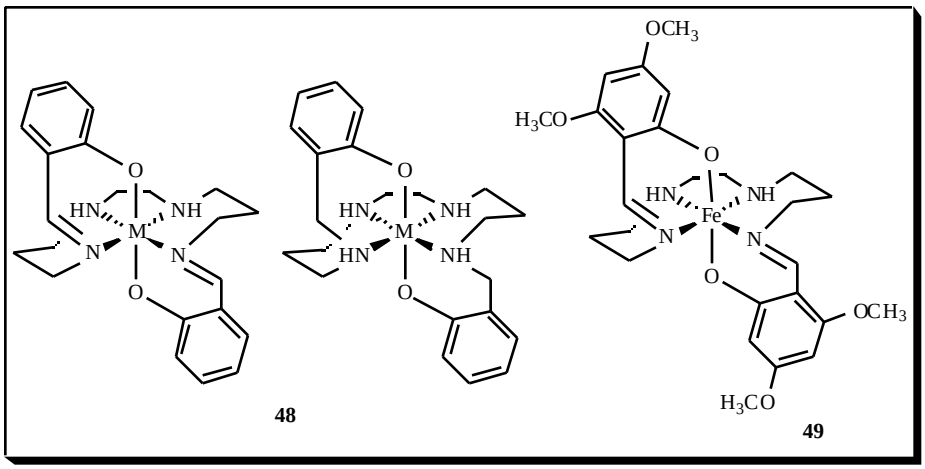
antimalarial compared with rhodium **43** and ruthenium **44** complexes against strains of both *P. berghei* and *P. falciparum* [94].

Based upon observations that non-iron porphyrins **46** have the ability to inhibit β-hematin polymerization via co-facial π-π interactions and potency of these derivatives increases in the presence of hydroxy groups, it has been postulated that compounds that possess these two characteristics may have better affinity for heme to participate in drug-heme complexes [95]. While bioavailability issues are under investigation, several metalloporphyrins **47** exemplified by M(III)PPIX (M= Cr(III), Co(III), Mn(II), Cu(II)) have shown ability to inhibit β-hematin formation and offer an interesting class of antimalarial drugs to study the process of hemozoin formation [96].

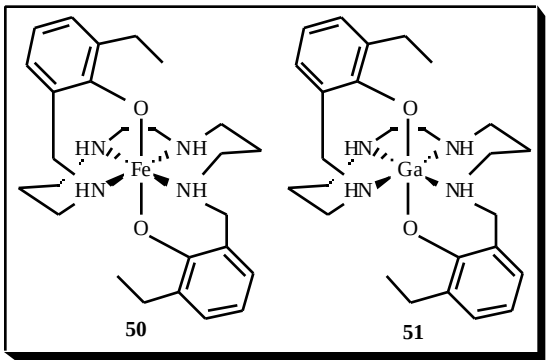
C. Metalloantimalarials

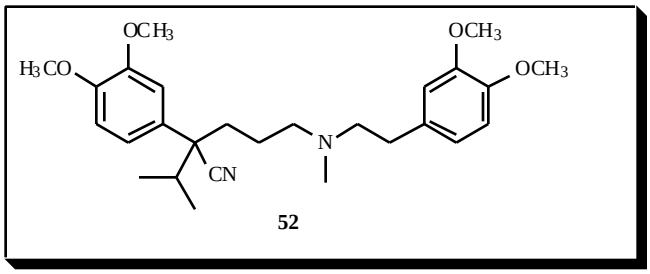
Metallo-drugs that possess an optimal balance of hydrophobicity/hydrophilicity, or relative lipophilicity, for permeation across membrane bilayers, and remain non-metabolized in the intracellular target sites, may offer alternative candidate drugs. Schiff-base phenolate- or amine phenol-metal complexes comprise another class of coordination compounds that possess favorable balance of hydrophobicity and delocalized cationic charge for enhanced cell membrane permeability [97]. Obtained from commercially available inexpensive precursors, these compounds have been found to possess antimalarial properties [98]. Further, organic ligands of **48** possessing six potential donor sites in their scaffolds are amenable to accommodating a variety of trivalent metals [99,100], including Al(III), Ga(III), In(III), and specifically biocompatible metals relevant to malaria-host interactions, such as Fe(III). The metal(III) complex **49** inhibited intraerythrocytic malarial parasitic growth in a stage specific manner. Inhibiting both chloroquine-sensitive and -resistant strains, efficacy of these metal complexes correlated with their ability to inhibit heme polymerization [98]. Further, Ziegler *et al.* have evaluated interactions of selected gallium(III) and iron(III) compounds with Fe(III)PPIX in the acetate buffer (pH 4.8) [101]. The results suggested that overall charge of the metal complex is a critical factor for determining activity for inhibition of hemozoin formation [101]. In addition, it has been shown earlier through SAR analysis that targeting properties of these metallo-drugs lie in the spatial orientation of the aromatic rings [97,102]. Thus, metallo-drugs target hemozoin formation by impeding propionate's ability to participate in the requisite axial linkages of the dimer, thereby inhibiting hemozoin formation [101].

Recently, while exploring structure-activity relationships by using gallium(III) as a surrogate metal for iron(III), we have found cationic, and moderately hydrophobic metallo-drugs, $[Fe\text{-}3\text{-}Eadd]^+$ **50** and $[Ga\text{-}3\text{-}Eadd]^+$ **51** that possessed antimalarial activity [103]. Those compounds, which



contain metals in octahedral environment, have been found to be stable under physiological conditions. Both **50** and **51** demonstrated promising half-maximum inhibitory concentration (IC_{50}) values of ~80 nM and 86 nM in the CQ-sensitive HB-3 line, respectively. However, both **50** and **51** have been found to possess elevated IC_{50} values of 2.5 μM and 0.8 μM, respectively, in the CQ-resistant Dd2 line (Fig. **4**), thus displaying preferential activity towards the CQ-sensitive HB3 line [103]. Both **50** and **51** targeted hemozoin formation in cultured parasites [103]. Thus, these compounds acted similar to chloroquine with regard to action and resistance, despite the lack of structural similarity to chloroquine [103].





CANDIDATE MECHANISM(S) OF CHLOROQUINE RESISTANCE IN MALARIA

Because chloroquine derivatives have been the therapeutic choice in treatment of malaria, herein brief discussion about mechanisms hypothesized to render these 4-amino-quinolines ineffective in *P. falciparum*, has been summarized below:

Seminal observations by Krogstad and colleagues demonstrated that the MDR reversal agent verapamil **52**, and the Pgp transport substrates daunorubicin and vinblastine enhanced the accumulation of chloroquine in drug resistant *P. falciparum* clones [104]. These observations suggested the existence of common resistance mechanisms in both malaria and cancer. The efflux rate of pre-accumulated chloroquine from resistant parasites was 40 times faster than the rate of efflux from sensitive parasites ($t_{1/2}$ = 2 min vs. 75 min) [104]. However, others demonstrated that the rate of drug efflux is a function of vacuolar concentration rather than the forces for either accumulation or efflux [105]. Earlier studies suggested gene amplification of *pfmdr* 1, an *MDR* 1 homologue, in chloroquine resistant isolates and none in chloroquine susceptible isolates [106]. Immunofluorescence and immunoelectron microscopy demonstrated expression of Pgh 1, the product of *pfmdr* 1, throughout the erythrocytic life cycle, and furthermore, in trophozoites, the protein was located predominantly on the vacuolar membrane [107]. The location was thought to be consistent with its putative role as a chloroquine transporter. However, quantification of Pgh 1 by immunoblot analysis did not show any correlation between overexpression and resistance and equivalent amounts were present in several chloroquine-sensitive and -resistant lines [107]. Subsequent efforts did not correlate *pfmdr* 1 gene with chloroquine resistance [108,109].

To further examine chloroquine resistance mechanisms, Wellems and coworkers performed a genetic cross between the chloroquine-susceptible HB3 clone and chloroquine-resistant Dd2 clone of *P. falciparum*. The cross generated

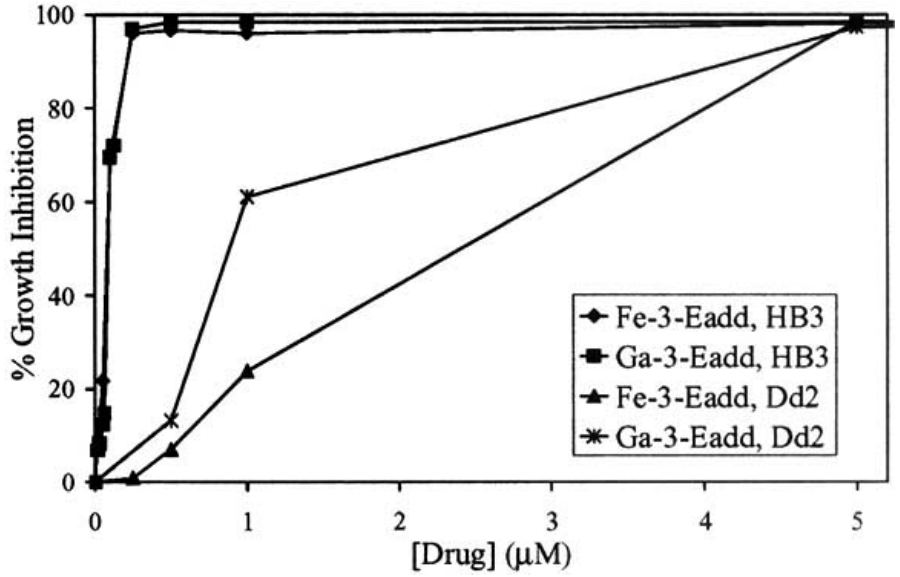
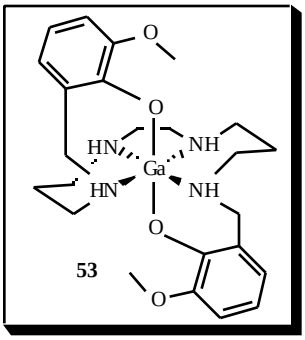


Fig. (4). Effect of **50** and **51** on intraerythrocytic *P. falciparum* in culture: CQ-sensitive (HB3) and -resistant (Dd2) lines were grown in the absence or presence of various concentrations of metalloantimalarials. Growth inhibition relative to control was measured by the ^{3}H-hypoxanthine incorporation assay. Data are shown as mean values of triplicate determinations.

independent offspring, which exhibited drug response characteristics of either the resistant or susceptible parent, suggesting a single genetic locus may be responsible for the drug susceptibility phenotype [110]. Analysis of restriction-fragment polymorphism enabled the parental origin of *pfmdr* 1 in the offspring to be identified. Further analysis of inheritance patterns demonstrated that the parental *pfmdr* 1 did not segregate with chloroquine-resistance. Thus, it was concluded that chloroquine-resistance is independent of MDR genes [110]. Further studies of genetic crosses using restriction fragment length polymorphism markers were able to distinguish an inheritance from chloroquine-resistant and chloroquine-sensitive parents [111]. An exhaustive search of the 14 chromosomes disclosed a perfect linkage of the chloroquine resistant phenotype to a genetic locus of around 36 kilobase on chromosome 7. This segment harbored eight potential genes, including the gene, *cg2* which encodes CG2, a unique ~330 kDa protein with complex polymorphisms [112]. Further examination of *P. falciparum* clones in transport experiments revealed that chloroquine accumulation was temperature-dependent, saturable, and inhibitable [113]. Kinetic analysis was consistent with these *P. falciparum* clones differentially encoding a protein that facilitates chloroquine import. The kinetics of chloroquine accumulation differ in isolates from both chloroquine sensitive and -resistant parasites derived from the genetic cross on the locus containing the 36 kilobase segment. Further analysis of these crosses showed competitive inhibition of chloroquine uptake by amiloride derivatives suggesting that chloroquine influx be mediated by a *Plasmodial* Na^+/H^+ exchanger [113]. While some sequence similarities in CG2 and Na^+/H^+ exchangers were identified [114], this had been refuted [115]. Further analysis of complex polymorphisms of *cg2* refuted it to be the chloroquine-resistant determinant. Instead an additional gene *pfcrt* (crt-chloroquine-resistance transporter) that is localized on chromosome 7 and encodes a transmembrane protein, PfCRT, has been shown to be the chloroquine-resistant determinant [116]. Wild-type PfCRT has been postulated to transport organic cations, and these profiles appear to be in accord with earlier postulation of a transporter-mediated efflux of basic amino acids that would otherwise accumulate in the digestive vacuole [117]. The mutant-type PfCRT has been found to possess a key mutation (Lys 76 changes into Thr 76) that determines CQR phenotype of *P. falciparum* in all malarial regions [118]. Alteration in mutant-PfCRT has been postulated to influence its ability to transport protonated amino acids, thereby resulting in decreased pH of the digestive vacuole. Thus, PfCRT has been postulated as a mediator of chloroquine-resistance in malaria [119].



53

While characterizing the antimalarial properties of Schiff-base phenolic complexes of Ga(III) and Fe(III) [98,102], an unusual selectivity profile was found with complexes of one particular amine-phenol ligand. This compound **53**, equipotent as the Ga(III) or Fe(III) complex, was shown to possess a pseudo-octahedral environment for the N_4O_2 donor core (Fig. **5**) and inhibited the same vital target as chloroquine, heme polymerization, with an IC_{50} of 0.5 μM. Curiously, in a genetic cross of independent recombinant progeny, sensitivity of this complex mapped in perfect linkage with the chloroquine-resistant phenotype (Fig. **6**) suggesting that a locus for susceptibility to this compound likely mapped to a gene of chromosome 7 as the chloroquine-resistance determinant [98].

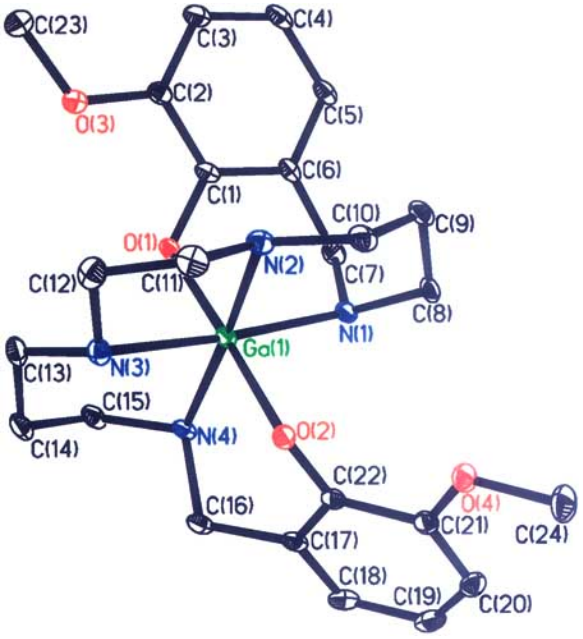


Fig. (5). ORTEP drawing of the 3-methoxy-ENBPA Ga(III) cation **53** that targets chloroquine-resistant clones of *P. falciparum*. Atoms are represented by thermal ellipsoids corresponding to 20% probability.

Recently, it has been shown that variation in the position of the methoxy moiety on aromatic rings of this ligand altered the cytotoxicity and specificity profiles for the compound, thereby metalloantimalarial **54** has been shown to be modestly active in the CQ-sensitive (HB3) strains only, and lacked the desired activity in the CQ-resistant (Dd2) strains (Fig. **7**) thus suggesting a targeted (e.g., transporter- or receptor-mediated) rather than non-specific (e.g., pH or other gradient-mediated) mechanism of action for these agents [120]. Nevertheless, these scaffolds offer an interesting template for development of antimalarial metal complexes that selectively target chloroquine resistance in malaria, and in addition, may be useful as novel probes of chloroquine resistance mechanisms in *P. falciparum*.

SUMMARY

Recent genomic discoveries of *Plasmodium falciparum* [3] and *Anopheles gambiae* [4] would provide enormous information about the interaction of the parasite with its host and carrier, including genes involved in parasite recognition

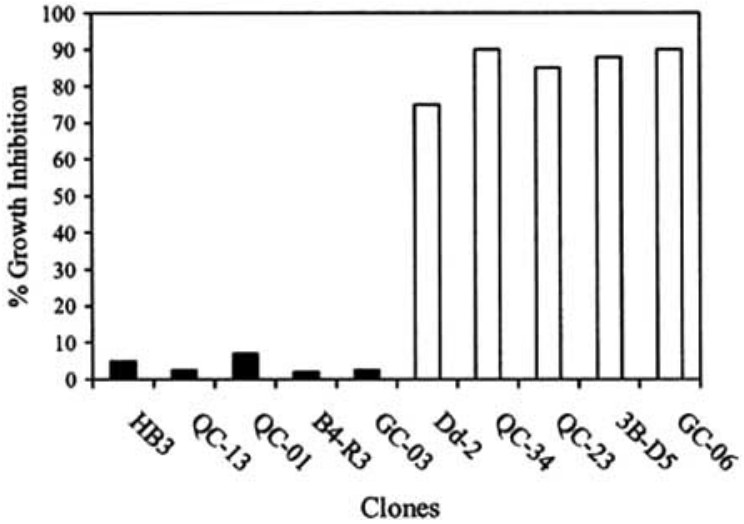
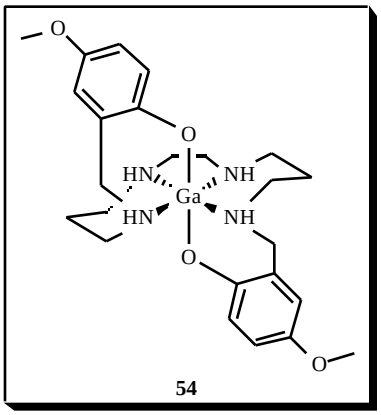


Fig. (6). Effect of 3-methoxy-ENBPA Ga(III) complex **53** on the growth of a *P. falciparum* genetic cross [selected clones from recombinant progeny; filled bars, CQ-sensitive clones; empty bars, CQ-resistant clones; controls, HB3 (CQ-sensitive); Dd2 (CQ-resistant)] in intraerythrocytic culture. Growth inhibition was measured by the ^{3}H-hypoxanthine incorporation assay. Data represent percent growth inhibition presented as mean values of 3-6 determinations.



54

by the human immune system. While translation of this vital information into effective remedies continues to be an area of intense investigation and new targets for drug development could emerge. Novel techniques for high throughput screening of combinatorial libraries also have emerged to further facilitate drug development [35]. The hydrophilic bis-artemisinin analogs that are extremely potent [59] and derivatized propane-1,3-diamine based hydrophobic compounds [35] that interfere with HRP II-mediated hemozoin formation offer promising leads for the future. Among selected targets that have been mentioned in this review, hemozoin formation continues to be a versatile target for drug development due to the selectivity of this process to parasitic growth versus the host. In addition, the *pfcrt* gene has been identified as a chloroquine- resistance determinant

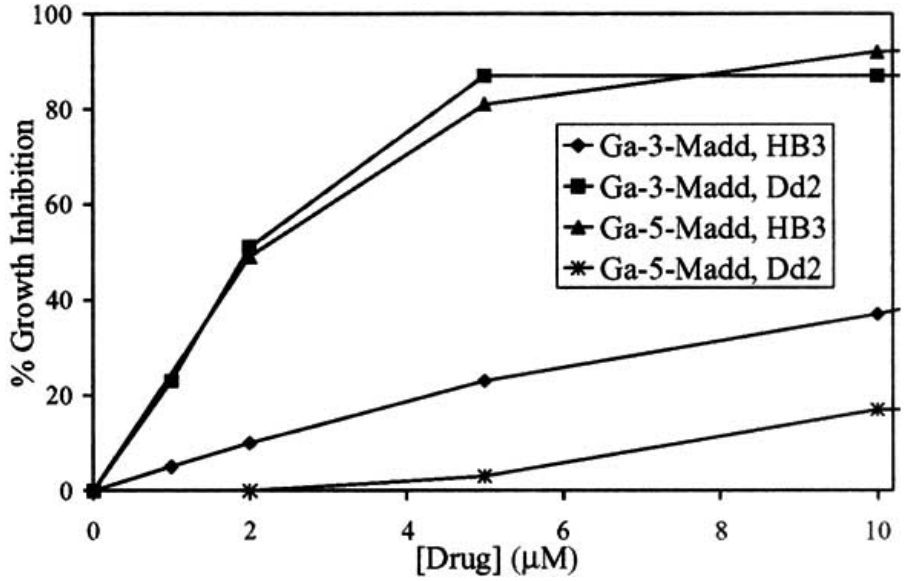


Fig. (7). Effect of **53** and **54** on intraerythrocytic *P. falciparum* in culture: CQ-sensitive (HB3) and -resistant (Dd2) lines were grown in the absence or presence of various concentrations of metalloantimalarials. Growth inhibition relative to control was measured by the ^{3}H-hypoxanthine incorporation assay. Data are shown as mean values of triplicate determinations.

[118]. However, existence of drug-resistance in malaria as a worldwide problem makes it mandatory for scientists to broaden the domain of available therapies well beyond the conventional purview of medicinal chemistry within the context of pharmaceutical research. The contribution of bioinorganic chemistry has started to make some impact. Thus, selected organometallic compounds, exemplified by ferrochloroquine derivatives, offer interesting therapeutic alternatives to chloroquine and its analogues in the chemotherapy of malaria due to their potency against both chloroquine-resistant and -sensitive strains both *in vitro* and *in vivo*. Furthermore, metalloantimalarials offer unique templates for examining the molecular mechanism(s) of chloroquine-resistance in *P. falciparum* strains. Despite the lack of structural similarities to quinoline-based antimalarials, selected analogs of metalloantimalarials target the same vital process, hemozoin formation in the heme-detoxification pathway, yet are able to evade CQ-resistance modes [102]. Thus, application of metallo-drugs in malaria promises to be an area of intense investigation into the future.

ACKNOWLEDGEMENTS

The author acknowledges help of all coworkers and collaborators (both past and present) for their contributions that are listed in this manuscript. In addition, author is grateful to colleagues, Prof. Daniel E. Goldberg and Prof. David Piwnica-Worms for their valuable suggestions during compilation of this review article. Financial assistance by the National Institutes of Health (R01AI45640) is gratefully acknowledged.

REFERENCES

[1] Woster, P. *Annu. Rep. Med. Chem.* **2001**, *36*, 99.
[2] Sturchler, D. *Parasitol. Today* **1989**, *5*, 39-40.
[3] Gardner, M.; Hall, N.; Fung, E.; White, O.; Berriman, M.; Hyman, R.; Cariton, J.; Pain, A.; Nelson, K.; Bowman, S.; Paulsen, I.; James, K.; Eisen, J.; Rutherford, K.; Salzberg, S.; Craig, A.; Kyes, S.; Chan, M.; Nene, V.; Shallom, S.; Suh, B.; Peterson, J.; Angiouli, S.; Pertea, M.; Allen, J.; Selengut, J.; Haft, D.; Mather, M.; Vaidya, A.; Martin, D.; Fairlamb, A.; Fraunholz, M.; Roos, D.; Ralph, S.; Mcfadden, G.; Cummings, L.; Subramanian, G.; Mungall, C.; Venter, J.; Carucci, D.; Hoffman, S.; Newbold, C.; Davis, R.; Fraser, C.; Barrell, B. *Nature* **2002**, *419*, 498-511.
[4] Holt, R.; Subramanian, G.; Halpern, A.; Sutton, G.; Charlab, R.; Nusskern, D.; Hoffman, S. *Science* **2002**, *298*, 129-149.
[5] Wirth, D. *Nature* **2002**, *419*, 495-496.
[6] Hastings, M.; Sibley, C. *Proc. Natl. Acad. Sci. U. S. A.* **2002**, *99*, 13137-13141.
[7] Ridley, R. *Proc. Natl. Acad. Sci. U. S. A.* **2002**, *99*, 13362-13364.
[8] Goldberg, D. *Science* **2002**, *296*, 482-483.
[9] Ridley, R. *Exp. Parasitology* **1997**, *87*, 293-304.
[10] Wendel, W. *Fed. Proc.* **1946**, *5*, 406-407.
[11] Korsinczky, M.; Chen, N.; Kotecka, B.; Saul, A.; Rieckman, K.; Cheng, Q. *Antimicrob. Agents Chemother.* **2000**, *44*, 2100-2108.
[12] Srivastava, I.; Rottenburg, H.; Vaidya, A. *J. Biol. Chem.* **1997**, *272*, 3961-3966.
[13] Srivastava, I.; Vaidya, A. *Antimicrob. Agents Chemother.* **1999**, *43*, 1334-1339.
[14] Egan, T.; Marques, H. *Coord. Chem. Rev.* **1999**, *190-192*, 493-517.
[15] Coombs, G.; Mottram, J. *Parasitology* **1997**, *114*, S61-S80.
[16] Rosenthal, P. *Adv. Parasitol.* **1999**, *43*, 105-159.
[17] Luker, K.; Francis, S.; Gluzman, I.; Goldberg, D. *Mol. Biochem. Parasitol.* **1996**, *79*, 71-78.
[18] Silva, A.; Lee, A.; Gulnik, S.; Majer, P.; Collins, J.; Bhat, T.; Collins, P.; Cachau, R.; Luker, K.; Gluzman, I.; Francis, S.; Oksman, A.; Goldberg, D.; Erickson, J. *Proc. Natl. Acad. Sci. U. S. A.* **1996**, *93*, 10034-10039.
[19] Haque, T.; Skillman, G.; Lee, C.; Habashita, H.; Gluzman, I.; Ewing, T.; Goldberg, D.; Kuntz, I.; Ellman, J. *J. Med. Chem.* **1999**, *42*, 1428-1440.
[20] Sijwali, P.; Shenai, B.; Gut, J.; Singh, A.; Rosenthal, P. *Biochem. J.* **2001**, *360*, 481-489.
[21] Bond, J.; Butler, P. *Annu. Rev. Biochem.* **1987**, *56*, 333.
[22] Troeberg, L.; Chen, X.; Flaherty, T.; Morty, R.; Cheng, M.; Hua, H.; Springer, C.; Mckerrow, J.; Kenyon, G.; Lonsdale-Eccles, J.; Coetxer, T.; Cohen, F. *Mol. Med.* **2000**, *6*, 660-669.
[23] Dominguez, J.; Charris, J.; Lobo, G.; Gamboa de Dominguez , N.; Moreno, M.; Riggione, F.; Sanchez, E.; Olson, J.; Rosenthal, P. *Eur. J. Med. Chem.* **2001**, *36*, 555-560.
[24] Eggleson, K.; Duffin, K.;Goldberg, D. *J. Biol. Chem.* **1999**, *274*, 32411-32417.
[25] Coombs, G.; Goldberg, D.; Klemba, M.; Berry, C.; Kay, J.; Mottram, J. *Trends in Parasitology* **2001**, *17*, 532-537.
[26] Muller-Eberhard, U.; Fraig, M. *Am. J. Hematol*. **1993**, *42*, 59-62.
[27] Viktoria, J.; Balla, J.; Yachie, A.; Akhiro, V.; Zsuzsa, V.; Gregory, M.; Eaton, J.; Balla, G. *Blood* **2002**, *100*, 879-887.
[28] Pagola, S.; Stephens, P.; Bohle, D.; Kosar, A.; Madsen, S. *Nature* **2000**, *404*, 307-310.
[29] Egan, T. *J. Inorg. Biochem.* **2002**, *91*, 19-26.
[30] Hempelmann, E.; Egan, T. *Trends in Parasitology* **2002**, *18*, 11.
[31] Egan, T. *Mini Rev. Med. Chem.* **2001**, *1*, 113-123.
[32] Wellems, T.; Howard, R. *Proc. Natl. Acad. Sci. U. S. A.* **1986**, *83*, 6065-6069.
[33] Panton, L.; McPhie, P.; Maloy, W.; Wellems, T.; Taylor, D.; Howard, R. *Mol. Biochem. Parasitol.* **1989**, *35*.
[34] Sullivan, D.; Gluzman, I.; Goldberg, D. *Science* **1996**, *271*, 219-221.
[35] Choi, C.; Schneider, E.; Kim, J.; Gluzman, I.; Goldberg, D.; Ellman, J.; Marletta, M. *Chem. & Biol.* **2002**, *9*, 881-889.
[36] Brinner, K.; Kim, J.; Habashita, H.; Gluzman, I.; Goldberg, D.; Ellman, J. *Biorg. Med. Chem*. **2002**, *10*, 3649-3661.
[37] Roberts, F.; Roberts, C.; Johnson, J.; Kyle, D.K.; Krell, T.; Coggins, J.; Coombs, G.; Milhous, W.; Tzipori, S.; Ferguson, D.; Chakrabarti, D.; McLeod, R. *Nature* **1998**, *393*, 801-805.
[38] McConkey, G. *Antimicrob. Agents Chemother.* **1999**, *43*, 175-177.
[39] Jomaa, H.; Weisner, J.; Sanderbrand, S.; Altincicek, B.; Weidemeyer, C.; Hintz, M.; Turbachova, I.; Matthias, E.; Zeidler, J.; Lichtenthaler, H.; Soldati, D.; Beck, W. *Science* **1999**, *285*, 1573-1576.
[40] Ralph, S.; D'Ombrain, M.; Mcfadden, G. *Drug Res. Updates* **2001**, *4*, 145-151.
[41] Woodward, R.; Doering, W. *J. Am. Chem. Soc*. **1944**, *66*, 849.
[42] Woodward, R.; Doering, W. *J. Am. Chem. Soc.* **1945**, *67*, 860.
[43] Sharma, V.; Piwnica-Worms, D. *Chem. Rev.* **1999**, *99*, 2545-2560.
[44] Yayon, A.; Cabantchik, Z.; Ginsburg, H. *EMBO J*. **1984**, *3*, 2695-2700.
[45] Sullivan, D.; Matile, H.; Ridley, R.; Goldberg, D. *J. Biol. Chem.* **1998**, *273*, 31103-31107.
[46] Hawley, S.; Bray, P.; Mungthin, M.; Atkinson, J.; O'Neill, P.; Ward, S. *Antimicrob. Agents Chemother.* **1998**, *42*, 682-686.
[47] Ziegler, J.; Linck, R.; Wright, D. *Curr. Med. Chem.* **2001**, *8*, 171-189.
[48] Foley, M.; Tilley, L. *Pharmacol. Ther.* **1998**, *79*, 55-87.
[49] Vippagunta, S.; Dorn, A.; Matile, H.; Bhattacharjee, A.; Karle, J.; Ellis, W.; Ridley, R.; Vennerstrom, J. *J. Med. Chem.* **1999**, *42*, 4630-4639.
[50] Mecozzi, S.; West, A.; Dougherty, D. *J. Am. Chem. Soc.* **1996**, *118*, 2307-2308.
[51] Kumpf, R.; Dougherty, D. *Science* **1993**, *261*, 1708-1710.
[52] Dougherty, D.; Stauffer, D. *Science* **1990**, *250*, 1558-1560.
[53] Bhattacharjee, A. *J. Mol. Struct.* **2000**, *529*, 193-201.
[54] Leed, A.; Dubay, K.; Ursos, L.; Sears, D.; Dios, A.; Roepe, P. *Biochemistry* **2002**, *41*, 10245-10255.
[55] Zhang, R. *Acta. Pharmaceutica Sinica* **1981**, *16*, 460-465.
[56] Posner, G. J. *Pharm. Pharmacol.* **1997**, *49* (Suppl 2), 55-57.
[57] Posner, G.; Oh, C.; Wang, D.; Gerena, L.; Milhous, W.; Meshnick, S.; Asawamahasakda, W. *J. Med. Chem.* **1994**, *37*, 1256-1258.

[58] Posner, G.; Cumming, J.; Ploypradith, P.; Oh, C. *J. Am. Chem. Soc.* **1995**, *117*, 5885-5886.
[59] Posner, G.; Paik, I.; Sur, S.; McRiner, A.; Borstnik, K.; Xie, K.; Shapiro, T. *J. Med. Chem.* **2003**, *46*, 1060-1065.
[60] Borstnik, K.; Paik, I.; Posner, G. *Mini Rev. Med. Chem.* **2002**, *2*, 573-583.
[61] Vennerstrom, J.; Eaton, J. *J. Med. Chem.* **1988**, *31*, 1269-1277.
[62] Dong, Y. *Mini Rev. Med. Chem.* **2002**, *2*, 113-123.
[63] Vennerstrom, J.; Fu, H.; Ellis, W.; Ager, A.; Wood, J.; Andersen, S.; Gerena, L.; Milhous, W. *J. Med. Chem.* **1992**, *35*, 3023-3027.
[64] Dong, Y.; Matile, H.; Chollet, J.; Kaminsky, R.; Wood, J.; Vennerstrom, J. *J. Med. Chem.* **1999**, *42*, 1477-1480.
[65] Solaja, B.; Terzic, N.; Pocsfalvi, G.; Gerena, L.; Tinant, B.; Opsenica, D.; Milhous, W. *J. Med. Chem.* **2002**, *45*, 3331-3336.
[66] Mabeza, G.; Loyevsky, M.; Gordeuk, V.; Weiss, G. *Pharmacol. Ther.* **1999**, *81*, 53-75.
[67] Hider, R.; Liu, Z. *J. Pharm. Pharmacol.* **1997**, *49*(Suppl. 2), 59-64.
[68] Pollack, S.; Rossan, R.; Davidson, D.; Escajadillo, A. *Proceedings Soc. Exper. Bio. Med.* **1987**, *184*, 162-164.
[69] Stahel, E.; Mazier, D.; Guillouzo, A.; Miltgen, F.; Landau, I.; Mellouk, S.; Beaudoin, R.; Langlois, P.; Gentilini, M. *Amer. J. Trop. Med. Hygiene.* **1988**, *39*, 236-240.
[70] Hershko, C.; Peto, T. *J. Exp. Med*. **1988**, *168*, 375-387.
[71] Gordeuk, V.; Thuma, P.; Brittenham, G.; Biemba, G.; Zulu, S.; Simwanza, G.; Kalense, P.; M'Hango, A.; Parry, D.; Poltera, A.; Aikawa, M. *Amer. J. Trop. Med. Hygiene.* **1993**, *48*, 193-197.
[72] Yinnon, A.; Theanacho, E.; Grady, R.; Spira, D.; Hershko, C. *Blood* **1989**, *74*, 2166-2171.
[73] Hershko, C.; Gordeuk, V.; Thuma, P.; Theanacho, E.; Spira, D.; Hider, R.; Peto, T.; Brittenham, G. *J. Inorg. Biochem.* **1992**, *47*, 267-277.
[74] Lytton, S.; Cabantchik, Z.; Libman, J.; Shanzer, A. *Mol. Pharma.* **1991**, *40*, 584-590.
[75] Lytton, S.; Loyevsky, M.; Mester, B.; Libman, J.; Landau, I.; Shanzer, A.; Cabantchik, Z. *Amer. J. Hematology* **1993**, *43*, 217-220.
[76] van Zyl, R.; Havlik, I.; Hempelmann, E.; MacPhail, A.; McNamara, L. *Biochem. Pharmacol.* **1993**, *45*, 1431-1436.
[77] Kung, H.; Molnar, M.; Billings, J.; Wicks, R.; Blau, M. *J. Nucl. Med.* **1984**, *25*, 326-332.
[78] John, C.; Costello, C.;Schlemper, E. *Polyhedron* **1992**, *11*, 2651-2655.
[79] Loyevsky, M.; John, C.; Zaloujnyi, I.; Gordeuk, V. *Biochem. Pharmacol.* **1997**, *54*, 451-458.
[80] Keberle, H. *Ann. N. Y. Acad. Sci.* **1964**, *119*, 758-768.
[81] Chevion, M.; Chuang, L.; Golenser, J. *Antimicrob. Agents Chemother.* **1995**, *39*, 1902-1905.
[82] Abrams, M.; Murrer, B. *Science* **1993**, *261*, 725-730.
[83] Wang, K.; Munoz, S.; Zhang, L.; Castro, R.; Kaifer, A.; Gokel, G. *J. Am. Chem. Soc.* **1996**, *1996*, 6707-6715.
[84] Plesske, K. *Angew. Chem.* **1962**, *1*, 312-327.
[85] Plesske, K. *Angew. Chem* **1962**, *1*, 394-399.
[86] Biot, C.; Glorian, G.; Maciejewski, L.; Brocard, J. *J. Med. Chem.* **1997**, *40*, 3715-3718.
[87] Domarle, O.; Blampain, G.; Agnaneit, H.; Nzadiyabi, T.; Lebibi, J.; Brocard, J.; Maciejewski, L.; Biot, C.; Georges, A.; Millet, P. *Antimicrob. Agents Chemother.* **1998**, *42*, 540-544.
[88] Biot, C.; Delhaes, L.; N' Diaye, C.; Maciejewski, L.; Camus, D.; Dive, D.; Brocard, J. *Biorg. Med. Chem.* **1999**, *7*, 2843-2847.
[89] Pradines, B.; Fusai, T.; Laloge, V.; Rpgier, C.; Millet, P.; Panconi, E.; Kombila, M.; Parzy, D. *J. Antimicrob. Chemother*. **2001**, *48*, 179-184.
[90] Biot, C.; Delhaes, L.; Maciejewski, L.; Mortuaire, M.; Camus, D.; Brocard, J. *Eur. J. Med. Chem.* **2000**, *35*, 707-714.
[91] Herde, J.; Lambert, J.; Senoff, C. *Inorg. Synth.* **1974**, *15*, 19.
[92] Sanchez-Delgado, R.; Navarro, M.; Perez, H.; Urbina, J. *J. Med. Chem.* **1996**, *39*, 1095-1099.
[93] McAuliffe, C.; Parish, R.; Randall, P. *J. Chem. Soc. Dalton Trans.* **1979**, 1730-1735.
[94] Navarro, M.; Perez, H.; Sanchez-Delgado, R. *J. Med. Chem.* **1997**, *40*, 1937-1939.
[95] Basilico, N.; Monti, D.; Olliaro, P.; Tarramelli, D. *FEBS Lett.* **1997**, *409*, 297-299.
[96] Cole, K.; Ziegler, J.; Evans, C.; Wright, D. *J. Inorg. Biochem.* **2000**, *78*, 109-115.
[97] Sharma, V.; Crankshaw, C.; Piwnica-Worms, D. *J. Med. Chem.* **1996**, *39*, 3483-3490.
[98] Goldberg, D.E.; Sharma, V.; Oksman, A.; Gluzman, I.Y.; Wellems, T.E.; Piwnica-Worms, D. *J. Biol. Chem.* **1997**, *272*, 6567-6572.
[99] Wong, E.; Liu, S.; Lugger, T.; Hahn, F.E.; Orvig, C. *Inorg. Chem.* **1995**, *34*, 93-101.
[100] Yang, L.W.; Liu, S.; Wong, E.; Rettig, S.J.;Orvig, C. *Inorg. Chem.* **1995**, *34*, 2164-2178.
[101] Ziegler, J.; Schuerle, T.; Pasierb, L.; Kelly, C.; Elamin, A.; Cole, K.; Wright, D. *Inorg. Chem.* **2000**, *39*, 3731-3733.
[102] Sharma, V.; Beatty, A.; Goldberg, D.E.; Piwnica-Worms, D. *J. Chem. Soc. Chem. Commun.* **1997**, 2223-2224.
[103] Harpstrite, S.; AA, B.; Collins, S.; Oksman, A.; Goldberg, D.; Sharma, V. *Inorg. Chem.* **2003**, *42*, 2294-2300.
[104] Krogstad, D.J.; Gluzman, I.Y.; Kyle, D.E.; Oduola, A.M.; Martin, S.K.; Milhous, W.K.; Schlesinger, P.H. *Science* **1987**, *238*, 1283-1285.
[105] Ginsburg, H.; Stein, W.D. *Biochem. Pharmacol.* **1991**, *41*, 1463-1470.
[106] Foote, S.J.; Thompson, J.K.; Cowman, A.F.; Kemp, D.J. *Cell* **1989**, *57*, 921-930.
[107] Cowman, A.F.; Karcz, S.; Galatis, D.; Culvenor, J.G. *J. Cell Biol.* **1991**, *113*, 1033-1042.
[108] Hyde, J. *Microb. Infect.* **2002**, *4*, 165-174.
[109] Ekong, R.M.; Robson, K.J.H.; Baker, D.A.; Warhurst, D.E. *Parasitology* **1993**, *106*, 107-115.
[110] Wellems, T.E.; Panton, L.J.; Gluzman, I.Y.; do Rosario, V.E.; Gwadz, R.W.; Walker, J.A.; Krogstad, D.J. *Nature* **1990**, *345*, 253-255.
[111] Wellems, T.E.; Walker-Jonah, A.; Panton, L.J. *Proc. Natl. Acad. Sci. U. S. A.* **1991**, *88*, 3382-3386.
[112] Su, X.; Kirkman, L.A.; Fujioka, H.; Wellems, T.E. *Cell* **1997**, *91*, 593-603.
[113] Sanchez, C.; Wunsch, S.; Lanzer, M. *J. Biol. Chem.* **1997**, *272*, 2652-2658.
[114] Sanchez, C.P.; Paul, H.; Lanzer, M. *Cell* **1998**, *92*, 601-602.
[115] Wellems, T.E.; Wootton, J.C.; Fujioka, H.; Su, X.; Cooper, R.; Baruch, D.; Fidock, D.A. *Cell* **1998**, *94*, 285-286.
[116] Fidock, D.; Nomura, T.; Talley, A.; Cooper, R.; Dzekunov, S.; Ferdig, M.; Ursos, L.; Sidhu, A.; Naude, B.; Deitsch, K.; Su, X.; Wootton, J.; Roepe, P.; Wellems, T. *Mol. Cell* **2000**, *6*, 861-871.
[117] Warhurst, D. *Parasitology today* **1988**, *4*, 211-213.
[118] Wellems, T. *Science* **2002**, *298*, 124-126.
[119] Sidhu, A.; D, V.-P.; Fidock, D.A. *Science* **2002**, *298*, 210-213.
[120] Ocheskey, J.; Polyakov, V.; Harpstrite, S.; Oksman, A.; Goldberg, D.; Piwnica-Worms, D.; Sharma, V. *J. Inorg. Biochem.* **2002**, *92*, 265-270.