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Current Status of Malaria Control

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1. Introduction:

Malaria remains one of the most important diseases of the developing world although it is known to humankind since ancient times in different forms and it is caused by Plasmodian malarial parasite. It kills approximately 1-3 million people and causes diseases in 300-500 million people annually. Pregnant women are the main adult risk group in most endemic areas of the world. [1-3] In Indian ancient literature Vedas it is described as a disease of high "*Jwar/ taap*" leading to death in most of the cases.[4] The disease poses a major public health challenges, which restrict the development in the poorest countries of the world.[5] The malaria parasite is a Plasmodian protozoan species, which evolved with time differentiating into four distinct species; *P. falciparum*, *P. vivex*, *P. malarae* and *P. ovale*, specific to man. Some other related species including *P. berghii* and *P. yeolii* are specific to other group of the mammalian class. The disease is transmitted from person to person through the bite of a female Anopheles mosquito.[6] Out of above four malarial parasites of human host, *Plasmodium vivax, P. malariae* and *P. ovale*, are the cause of intermittent high fevers making a person very ill but they are rarely fatal. The remaining species *P. falciparum*, is the cause of malignant tertian, or *falciparum*, malaria which has a substantial mortality if it is untreated, especially in the first or an early attack.[7-10] Following a single infection, there is intermittent presence of the parasites in the blood for several months. In population of frequent mosquito bites, the parasitaemia continues throughout childhood.[11-13]

2.Pathogenesis and Life Cycle of the malaria parasite:

The infected primary host, the female anopheles mosquito, injects sporozoites into secondary (human) host at the time of blood suck. The sporozoites migrate to the liver and invade hepatocytes within 1 h. where they complete the pre-erythrocytic and exo-erythrocytic stages of their life cycle leading to hepatic schizogony.[14,15] After 5–7 days, the infected hepatocytes rupture and release thousands of merozoites, which invade erythrocytes and start the erythrocytic phase.[16,17] The parasite develops and replicates within the erythrocytes and after 24–26 h in the cycle the trophozoite adheres to the endothelium of small blood vessels. This adherence (sequestration) is the cause of the pathophysiology of falciparum malaria. In this period (26–48 h) the older stages (merozoits) cannot

normally be seen in the blood smear. The trophozoites grow to the schizonts (erythrocytic schizogony) and after 48 hrs rupture the erythrocytes release their progeny (16–32 merozoites per schizont) in the blood.[18, 19] An unidentified malaria toxin is released on rupture of schizont-erythrocyte resulting in cytokine response which leads to clinical manifestations of the typical malaria inluding high fever, chills, prostration and anaemia. Severe disease can include delirium, metabolic acidosis, cerebral malaria and multiorgan system failure, and comma and death may ensue. The period between the mosquito bite and the appearance of the first symptoms is called the incubation period which is specific for the four species of plasmodium.The pathogenicity of the parasite results because of its rapid rate of asexual reproduction in the host and its ability to sequester in small blood vessels. [17]

3. Traditional and Current Approaches over Malaria Control :

Both the traditional and current approaches have been used to control malaria The use of impregnated bed-nets with residual pyrethroids, e.g. perimethrin and deltamethrin, is likely to increase, once their value in reducing malarial morbidity is more widely established. It has been shown that the success of bed net programme is dependent on a variety of factors - vector susceptibility to pyrethroids, high coverage of the population at risk, high malaria incidence, good community participation, high mosquito densities when people go to bed and a high prevalence of *P. falciparum.* [20-22]

The current approaches to curtail this disease include the vector control, immunotherapy, vaccination and the chemotherapy.

3.1. Vector Control:

Vector control can be achieved either by making contact of human host and mosquito host impossible or killing the mosquitoes by insecticides.[23] In the context of the vector control the female anopheles mosquito may be considered as the moving targets and thus control may be broadly divided into three main categories (a) reducing vector density (b) interrupting their life cycle, and (c) creating a barrier between the human host and the vector, i.e. simply preventing the mosquito bite.

The environmental modification/manipulation and changes in the biosystem are solutions to control vector density.[24] Interruption of the life cycle leading to eradication of mosquito host includes

destroying their breeding sites and resting areas and more specifically use of organisms feeding on vector larvae. Artificial barrier between the vector and the host can be met by using the insecticides, repellents, protective clothing's and the bed nets. Although the vectors in many parts of the world are still susceptible to pyrethroids and carbamates, yet it got a setback due to development of resistance against insecticides and the entry of insecticides in the human food chain leading to various serious complications.[25]

3.2. Vaccination:

Vaccination is an attempt to mimic certain aspects of an infection leading to an immune response that will protect the individual from that infection. Vaccination in malaria also like many other diseases represents one of the most important approaches that would provide a cost-effective intervention in addition to currently available malaria control strategies.[26] During the past decade understanding the immune mechanism involved in the protection against this disease has made significant progress and many vaccine candidate antigens and their genes have been identified.[27]

An ideal malaria vaccine encompasses mainly three essential characteristics; (a) it is multi-stage, incorporating antigenic characteristics at multiple stages of *P. falciparum*'s life cycle, (b) it is multi-valent, containing multiple epitopes restricted by different MHC molecules, which would help in overcoming the genetic restriction and allelic and antigenic variations, and (c) it is a multi-immune, inducing more than one type of immune response, comprising both cell-mediated and humoral immunity. Such a multi-component vaccine would increase the probability of a more sustainable and effective host response

Most of the vaccine trials were directed against liver stages or sporozoites, and these vaccines included completely synthetic peptides, conjugates of synthetic peptides with proteins such as tetanus-toxoid to provide Helper T-cell, recombinant malarial proteins, particle-forming recombinant chimeric constructs, recombinant viruses, and bacteria- and DNA-based vaccines.[28] Asexual blood stage vaccine trials have used either synthetic peptide conjugates or recombinant proteins. Only one trial, so far has been conducted for transmission-blocking vaccine using recombinant Pfs258. Some of recently developed vaccines against falciparum malaria are discussed below.

1. SPf66: It is the first recognized malaria vaccine [29] developed in Colombia by Manual Patarroyo in 1987 from three merozoite-derived proteins by joining them with sequences derived from the repeat domain of the circumsporozoite (CS) protein of *P. falciparum*. Phase I trials demonstrated a 75% rate of efficacy and showed that the vaccine was immunogenic and well tolerated. Phase IIb and III trials demonstrated an efficacy rate of 38.8-60.2%. The first trial in Africa was conducted in Tanzania in 1993, where intense malaria transmission occurs. The estimated vaccine efficacy rate was 31% after a one-year follow-up period. SPf66 was also confirmed to be safe and immunogenic. A later trial in the Gambia did not show any protective effect elicited by the SPf66 vaccine; however, factors such as the short three and a half months follow-up period were given as reasons for this outcome.

However, recent studies have shown that this vaccine has very low immunogenicity and induces only a temporary humoral immune response (6 months on average).

2. CSP [30a]**:** It is a circumsporozoite protein (CSP) incorporating the recombinant (Asn-Ala-Asn-Pro15-Asn-Val-Asp-Pro)2-Leu-Arg (R32LR) covalently linked to purified Pseudomonas aeruginosa toxin A 9. In contrast to anticipation neither there was an elevation of T-lymphocyte response nor the reduction in incidence of disease in the study group as compared to the control group (which received hepatitis B vaccine). CSP vaccine recipients had an 82% incidence of parasitemia, while the control group had an 89% incidence, indicating that CSP vaccine-induced anti-sporozoite antibody was not protective. Furthermore, DNA vaccines against malaria are known to have CSP sequencing genes.

3. NYVAC-Pf7 [30b]**:** It is a recently developed multistage vaccine and is a single NYVAC genome containing genes encoding seven antigens from *Plasmodium falciparum*. Out of these seven antigens, two are derived from the sporozoite stage of the parasite life cycle (CSP and sporozoite surface protein 2 (PfSSP2)), one from the liver stage (liver stage antigen 1 (LSA1)), three from the blood stage (merozoite surface protein 1 (MSP1), serine repeat antigen (SERA), and AMA-1), and one from the sexual stage (25-kDa sexual- stage antigen (Pfs25)). The purpose of this multiple antigens vaccine is to induce immunity in recipient. Trials with *Rhesus monkeys* were carried out with promising results where specific antibody responses against four of the seven antigens were observed. These were the

CSP, PfSSP2, MSP1, and Pfs25. The main objective for the use of a poxvirus-based vaccine was to maximize the elicitation of cellular immunity.

In phase I/IIa safety, immunogenicity and efficacy vaccine trials in humans (1998), vaccine was tolerated by variably immunogenic. Antibody responses were generally poor; however, cellular immune responses were detected in well over 90% of the test subjects. In some cases, administration of the vaccine conferred complete protection to falciparum challenge.

4. [NANP]19-5.1[31]**:** It consists of 19 repeats of the sporozoite surface protein [NANP] and the schizonts export antigen 5.1. A field trial with the recombinant *P. falciparum* vaccine [NANP]19-5.1 yielded promising results. However, this vaccine has limitation of containing no immunodominant T-cell epitopes. In its current form, the vaccine is only 20% peptide and has limited immunogenicity. The use of recombinant IL-2 adjuvant in conjunction with this vaccine has proven to be successful.

5. RTS, S [32]**:** It is a recombinant vaccine consisting of the circumsporozoite surface protein of the sporozoite stage of *Plasmodium falciparum*. This antigen elicits antibodies that are capable of preventing sporozoites from invading hepatocytes, and a cellular response that is capable of eliminating infected hepatocytes. The problem with the circumsporozoite protein is that it is poorly imunogenic. Therefore, in the RTS, S vaccine, the circumsporozoite protein is fused with a hepatitis B surface antigen, to create a much more potent vaccine. This vaccine was combined with both alum and monophosphoryl A or only oil in water emulsion, and proved to be effective.

A human subject study concluded that in addition to superior humoral responses, vaccinated individuals demonstrate extreme T-cell proliferation and IFN-gamma production. The current limitation is that conferred immunity rapidly declines following a six-month period. The improvement of vaccine composition, use of adjuvants, and an improved immunization schedule offer hope to confer a long-lasting protective immunity.

6. Pfs230 [33]**:** It is a sexual-stage falciparum xsurface antigen and can elicit antibodies which block the infectivity of gametes to mosquitoes. The 360-kDa protein is localized to the

parasitophorous vacuole/parasite plasma membrane. I t has been shown that sera which mediate gametolysis contain IgG1 and IgG3 antibodies to gamete surface proteins. Thus, Pfs230 is a major target of C'-fixing antibodies.

7. DNA Vaccines[34]:

DNA based vaccines are the newest technology that may hold the key to control many infectious diseases. DNA vaccine is source of a stable and long-lived protein vaccine which can induce both antibody- and cell-mediated immune responses to a wide variety of antigens. In vaccination DNA enters the host cells and makes the proteins which stimulate the immune system to produce antibodies and cellular (T-cell) responses that may prevent or control the malaria. Moreover, the flexibility of DNA vaccine technology permits the combination of multiple antigens from both the pre-erythrocytic and erythrocytic stages of malaria parasite. For DNA and recombinant virus subunit vaccines, the DNA sequence for the antigen(s) of choice are inserted into an *E. coli*-derived purified plasmid or the genome of a double-stranded DNA virus such as vaccinia.The host CD4+ and CD8+ responses can then be induced following intracellular synthesis, processing and HLA (Human Leukocyte Antigen) presentation of class I and II T cell epitopes.[33] Many DNA vaccines with genes coding for different antigenic parts of malaria proteins have been created and presently some of these are undergoing field trials. It is very easy and cheap to make DNA vaccine than other vaccines made from conventional proteins. Further, these vaccines don't need refrigeration, and are responsive to genetic manipulation. Several DNA vaccines are in clinical trial and are effective in animal models. The results from these trials will help to determine the likelihood of success of this technology in humans.

3.3. Immunotherapy [35--42] and Genomics [43-46]:

Immunity development against malaria is very complex phenomenon in individuals living in areas of high endemicity. People residing in malaria endemic regions, over long periods of inhabitation/exposure, naturally acquire protective immunity against the disease, although the patterns of immunity vary with malaria transmission patterns. Several studies have demonstrated that purified immunoglobulins from the sera of immune adults living in endemic regions, and of experimentally immunized animals can passively transfer protection against a live challenge. Clinical studies have

demonstrated that experimental vaccination of humans with attenuated sporozoites can induce effective protection against a subsequent challenge. [35] Animal studies of malaria vaccination clearly demonstrated the potential for the induction of protective immunity, following active immunization using different *Plasmodial* components for e.g., immunization with *P. knowlesi* Mz13 can induce immunity, which has been found to be superior to the immunity developed from natural infections in humans.

Antibody-dependent mechanisms are presumed to play an important role in protection, with a wide range of antigen-specific antibodies as well as polyclonal-antibody production. It is known that IgG antibodies are important in reducing parasite density during this disease

Genetic mapping of P. falciparum has recently been revealed.[46] It is known that the parasite has 14 chromosomes, approximately 5300 genes responsible for protein synthesis are known. Two third of the total genes are unique to the parasite. Further about 208 genes are known to be responsible for the evasion of parasite from host immune response.

3. 5. Chemotherapy:

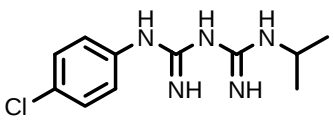
The chemotherapy of malaria basically involves killing of the asexual parasites in the circulation (the sexual forms are important for the spread of drug resistance and are non-pathogenic) and providing supportive therapy to the host to boost the immune system. [47-50] Antiinfective drugs rely their efficacy and specificity on their ability to interfere with the aspects of metabolism that differ significantly from the human host. During the life cycle of the parasite in human erythrocytes the Plasmodium parasite requires several metabolic adaptations and innovations that render it susceptible to chemotherapeutic attack.[51]The parasite degrades hemoglobin in its acidic food vacuole producing free heme which react with molecular oxygen and generate reactive oxygen species as toxic by-products. A major pathway of detoxification of heme moieties is polymerization of heme to heamazoin. Majority of antimalarial drugs act by disturbing the polymerization (and /or the detoxification by any other way) of heme, thus killing the parasite with its own metabolic waste.[51] A series of reports exist for different drugs and their efficacy against malaria. [52-55]

4. Validated Biochemical targets to Develop Drugs:

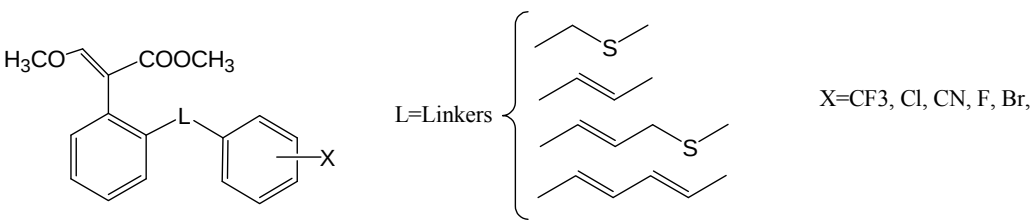
Before discussing the drugs or their combination to curtail this disease, it is appropriate to look upon the main biochemical targets, being utilized in developing new drugs.

4.1 Mitochondrial electron transport:

The unusual morphology of the plasmodium mitochondrian is a very good target for the antimalarial drug development.[56] The parasite resides in an oxygen deficient environment and is totally dependent on the glycolytic pathway for its ATP. The mitochondrion of the parasite lacks the oxidative phosphorylation, and an incomplete electron transport chain is the source of the certain metabolically significant redox reactions. [57] Coupling of cytochrome C reductase to dihydroorotate degydrogenase, a key enzyme in nucleoside biosynthesis is crucial for malarial parasite.[58] Thus inhibitors of cytochrome C reductase are the possible antimalarial agents. β-methoxy acrylates and proguanil are known inhibitor of this enzyme.[59, 60]



Proguanil



β-Methoxy Acrylates

4. 2. Glycolytic pathway:

It is known that in the plasmodium infected erythrocytes the glucose consumption is 50-100 fold higher than the normal ones.[61] The parasite requires enormous amount of glucose uptake from the

host to support its growth.[62] Although many glycolytic enzymes from *P. falciparum* have been cloned and sequenced yet only two enzymes TIM(Triosephosphate isomerase) and Lactate dehydrogenase (LDH) have been seriously thought to develop new antimalarial aagents. Triosephosphate isomerase (TIM) is a dimeric glycolytic enzyme, which catalyzes the isomerization of -glyceraldehyde-3-phosphate to dihydroxyacetone phosphate has been crystallized and its implication in new malaria control has been discussed in detail.[63]

Lactate dehydrogenase (LDH), the last enzyme in glycolysis, is apparently essential for the survival of Plasmodium falciparum and therefore it can be used as target for developing new antimalarial agents. In the crystal structure of the parasitic LDH, a distinctive cleft has been discovered.[67]

4. 3. Folate pathway:

In all species of the malaria parasite the pyrimidine biosynthesis is known to be *de novo*. Dihydroorotate dehydrogenase (DHODH) is localized in the mitochondria and catalyzes the rate limiting step of UMP formation in the pyrimidine biosynthetic pathway. [65-67] Dihydroorotate dehydrogenases catalyzes the oxidation of dihydro orotate to orotate utilizing the flavin cofactor (FMN). In the second step, the enzyme catalyzes the reoxidation of FMNH by using respiratory chain type quinones (coenzyme Q). All the cytoplasmic forms of dehydrogenases oxidize FMNH 2 through NAD+ or fumarate, while membrane or mitochondrial forms require respiratory quinones.[66] Sequence analysis of malarial DHODH gene reveals that it belongs to the mitochondrial type membrane enzyme. Since the erythrocytic stages of the Plasmodium parasites lack a number of enzymes of the tricarboxylic acid (TCA) cycle, the electrons from reduced quinones generated from DHOD reaction, are funneled to the electron transport chain of the parasite respiratory pathway.[67] Therefore, the inhibitors of the enzymes (different from human host) involved dihydrofolate synthesis would prove to be good antimalarial agents.

4. 4. Proteases & heme metabolism:

The parasite degrades up to 80 % of the host cell haemoglobin in lysosomal food vacuole[68] and involves aspartic proteases (plasma pepsin)[69] the cystein protease, falcipain 2[70] and many peptidases including metallopeptidases[71] in erythrocytic life cycle, which is responsible for all clinical

manifestations of malaria, begins when free merozoites invade erythrocytes. The intraerythrocytic parasites develop from small ring-stage to larger, more metabolically active trophozoites and then to multinucleated schizonts.[72] The erythrocytic cycle is completed when mature schizonts rupture the erythrocytes, releasing numerous invasive merozoites. A number of malarial proteins are proteolytically processed during the late schizont and merozoite stage. Merozoite surface protein-1 is processed in a manner inhibited by serine protease inhibitors presumably to facilitate the complex series of events involved in erythrocyte. The proteases are required for the rupture and subsequent re-invasion of erythrocytes by merozoite and for the degradation of hemoglobin by intraerythrocytic trophozoites and thus these are among potential targets for the development of new antimalarial agents.[73-74]

The degradation of hemoglobin is a source of the free amino acids for the protein synthesis in malarial parasites.[75] The heme is a major component of malarial pigment heamazion,[76] and globin. The latter is hydrolyzed to its constituent amino acids lysosomal proteases from the food vacuole, an acidic organelle analogous to lysosomes. Several of such proteases have been characterized, e.g. cysteine (cathepsins B, H, and L) and aspartic (cathepsin D) haemoglobin. At least two aspartic proteases and one cysteine protease have been isolated from purified *P. falciparum* food vacuoles .[77-78]

Antimalarial drugs appear to act by preventing hemozoin formation, producing free radicals in the food vacuole or, in the case of experimental compounds, preventing globin hydrolysis. The 4-aminoquinolines (chloroquine) and aryl alcohols [79-83] and artemesinin or its analogs [84,85] appear to act by blocking the formation of hemozoin from heme molecules once they are liberated from hemoglobin and the antiparasitic effects are presumably engendered by the toxicity of free heme, possibly by disruption of membranes.

4. 5. Apicoplast metabolism:

Elucidation of apicoplast metabolism, has significant implications in the parasite life cycle and this plastid like orgenelle is the target of many clinically used antimalarial antibiotics. The orgenelle has a 35 kilobase circular genome including elements of a prokaryotic translation and transcription system, and it has been shown that malaria parasites are susceptible to prokaryotic transcriptional inhibitors such as rifampicin and DNA gyrase inhibitors such as quinolones.[86]

Out of many targets from the apicoplast of the malarial parasite the two very important targets are Type-II fatty acid biosynthesis (FAS-II) and nonmevalonate pathway leading to synthesis of isopentenyl diphosphate subunits.[87,88] Disruption of FAS-II results in rapid cell death. Thiolactomycin [89] inhibits this enzyme and is known to inhibit *P. falciparum* growth also. Another inhibitor Triclosan [90] is active against paarsite growth in cell culture and in rodents model through inhibition of enoyl-acyl carrier protein reductase. Fosmidomycin having antimalarial activity is known to inhibit 1-deoxy-D-xylo-5-phosphate synthase (DOX-5) and offers a lead molecule for antimalarial drug development. [91-92]

4. 6. Glycophospatidyl inositol (GPI) as a Target:

Differences in the susceptibility to the inhibitors of the GPI biosynthesis have been described for protozoan, yeast and mammalian cells opening the door for the development of specific inhibitors of GPI biosynthesis as new therapeutic agents.[93-96] It has been speculated that detailed studies on GPI biosynthesis in Plasmodium might led to the identification of parasite specific reaction of this pathwayparticularly the inositol myristoylation is a unique feature of plasmodial GPIs and thus might provide a potential target for drug therapy. [97]

4. 7. Lipid metabolism (Glycerophospholipids):

Glycerophospholipids (GPLs) are essential components of numerous membranes in all living organisms. Two major GPLs in *P. falciparum*, *viz*. phosphatidylcholine (PtdCho) and phosphatidylethanolamine (PtdEtn) make up around 80% of the total phospholipid content. [98] Other GPL species associated with parasite cells, which constitute a significant proportion of total phospholipids, are phosphatidylserine (PtdSer), phosphatidylinositol and cardiolipin, though the content of these three species is sevenfold less than that of phosphatidylcholine and phosphatidylethanolamine. [99] The growing parasite requires large amounts of lipids for increase in parasite surface area and volume of internal membranes. This huge demand for lipids makes lipid metabolism an attractive target for anti-malarial drugs and several potential drugs targeting lipid metabolism have been identified [100]

Several parasite enzymes involved in lipid synthesis from glycerides and fatty acids, as well as enzymes involved in the remodeling of lipid polar head groups have been identified. [100] An enzyme capable of activating fatty acids (necessary for incorporation into lipids) has been localized to membranous structures found within the cytoplasm of the infected erythrocyte[101]

4. 8. Peptidyl deformylase as target:

Peptidyl de formylase is a metalloenzyme which has recently been recognized to utilize Fe^{++} (Iron-II) as the catalytic metal for *N*-formyl hydrolysis during peptide biosynthesis. PDF removes the formyl group from the N-terminal methionine of nascent polypeptides, represents an interesting and unexploited target [102,103] since this enzyme is conserved and believed to be essential in plasmodium falciparum & eubacteria but not required for protein synthesis in Human.[104] Several hydroxamate based compounds are reported to be inhibitor of the peptide deformylase[105] and very recently we have synthesizes glycosyl hydroxamates and few of them showed in vitro antimalarial activity.[106]

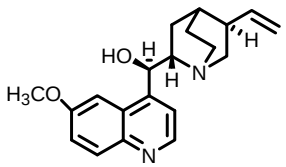
4. 9. Oxidative stress in the infected erythrocytes:

For survival and proper growth of the malaria parasite a redox system is needed for the protection of the parasite from the reactive oxygen intermediates (ROI) (superoxide, hydroxyl radical and hydrogen peroxide generated by metabolism) as these ROI are lethal to the parasite. Redox metabolism involving superoxide dismutase (SOD), catalase, and glutathione peroxidase are involved in the detoxification of ROI. It has also been proposed that the parasite uses host catalase and SOD within the food vacuole. Many enzymes involved in redox metabolism are known and out of them glutathione reductase has been identified as potential antimalrial drug target and many inhibitors of this and other enzymes are known to act as very good antimalarial. [107-109]

5. Current chemotherapy of Malaria:

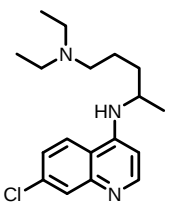
5.1 The most commonly used drugs in single drug therapy for the early diagnosed malaria are given below.

Quinine[110]**:** Isolated from the bark of Cinchona tree it is the only drug, which over a long period of time, has remained largely effective in treating the disease. A number of its derivatives are known to be good antimalarials. However, It is now only used for treating severe falciparum malaria, partly because of undesirable side effects[111-113]



Quinine

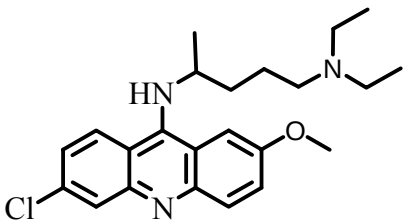
Chloroquine[114]**:** It is an effective and cheap drug both from prophylactic and chemotherapeutic view point. It was first used in the 1940s shortly after the Second World War and was effective in curing all forms of malaria, with few side effects when taken in the dose prescribed. Unfortunately, most strains of falciparum malaria are now resistant to chloroquine and more recently chloroquine resistant vivex malaria has also been reported.[115]



Chloroquine

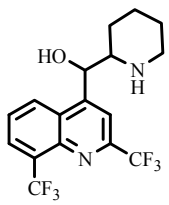
Alebrin (Mepacrine):

This drug was developed in the early 1930s and used as a prophylactic on a large scale during the Second World War (1939-45). It had a major influence in reducing the incidence of malaria among the troops serving in Southeast Asia. Because of its many undesirable side effects it is no longer used nowadays. [116]



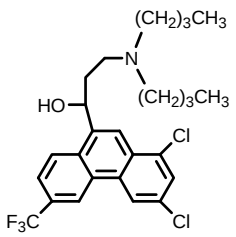
Alebrin/ Mapacrine

Mefloquine (Lariam, 1971): Structurally related to quinine, this drug was effective against many resistant malaria starins. Initially it was considered as good prophylactic because of its long half life. Widespread resistance and undesirable side effects (mainly the acute brain syndrome) associated with this drug have resulted in a decline of its use. [117-118] Because of its relationship to quinine the two drugs must not be used together.



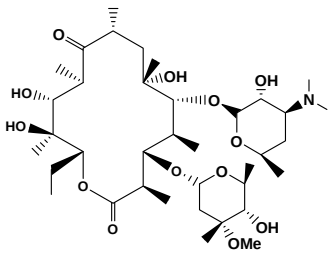
Mefloquine

Halofantrin (Halfan1980s): It is is an effective antimalarial drug but due to its short half life of 1 to 2 days and its high cost, it is not suitable for use as a prophylactic. [119-120] Unfortunately, resistant forms are increasingly being reported and there is some concern about its side effects. Halofantrin has been associated with neuropsychiatric disturbances. It is contraindicated during pregnancy and is not advised to women who are breastfeeding. Abdominal pain, diarrhea, purists and skin rash are some of the common side effects [121]



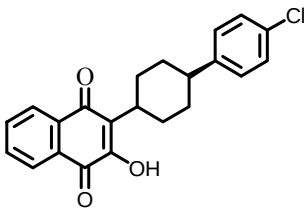
Halofantrin

Azithromycin [122]**:** It is mainly used for the chemoprophylaxis. It also shows limited toxicity but the studies are limited to date.



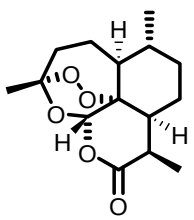
Erythromycin

Atovaquone[123]**:** It is an important antifolate drug for malaria treatment and is used in combination with proguanil which a prodrug and metabolically converted to cycloguanil an antifolate.



Atovaquone

Artemisinin [124]**:** It was derived from a Chinese herbal remedy and covers a group of products. The two most widely used are artesunate and artemether. While they are widely used in Southeast Asia, they are not licensed in much of the Western World. A high rate of treatment failures with drug led to a combination regimen with mefloquine for the treatment of falciparum malaria.

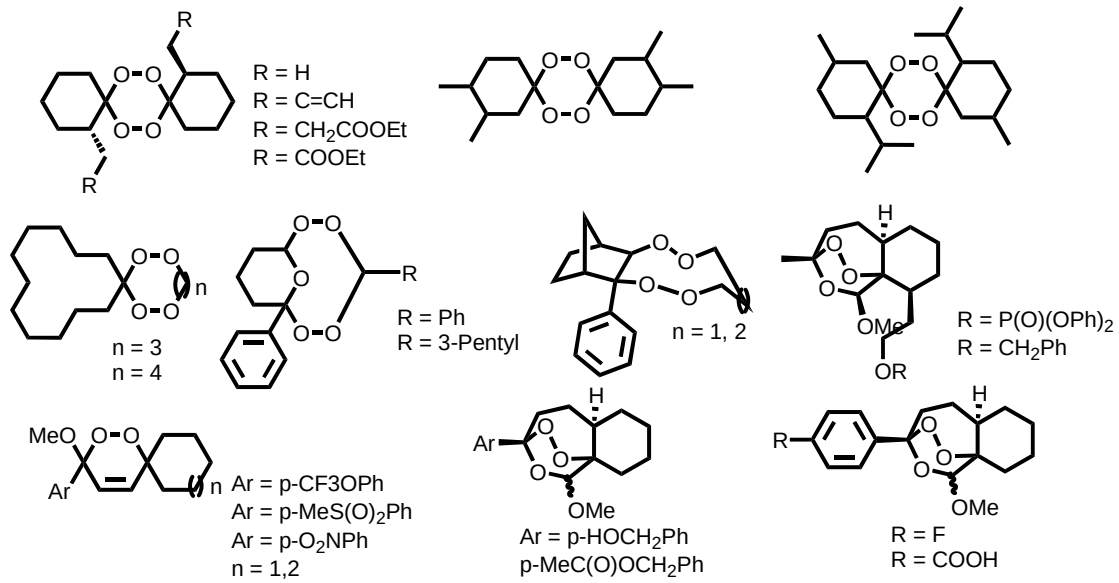


Artimisinin

Peroxides in Malaria treatment [125-131]**:**

Artemisinin derivatives are the fastest active antimalarial drugs. Four compounds have been used, the parent one, artemisinin, extracted from *Artemisia annua* and three derivatives that are actually more

active than artemisinin itself. One of them is a water-soluble hemisuccinate: artesunate; two others are oil-soluble ethers: artemether and arteether.



Peroxides as antimalarial drugs

Artemisinin is active at nanomolar concentrations *in vitro* on both CQ-sensitive or -resistant *P. falciparum* strains. Artemisinin and its derivatives appear to be the best alternative for the treatment of severe malaria and artemether has been included in the WHO List of Essential Drugs for the treatment of severe MDR malaria. In this family, the Walter Reed Institute of Research has patented a stable, water-soluble derivative calledartelinic acid that is now being tested in animals. A key advantage of these endoperoxide-containing antimalarial agents, which have been used for nearly two decades, is the absence of any drug resistance.

A low level of resistance has been observed, which disappeared as soon as the drug-selection pressure has been withdrawn. Artemisinin derivatives being gametocidal reduce the transmission of the disease. However, drawback of artemisinin derivatives is their short half-life (3–5 h). Combination of peroxides with other drugs in malaria chemotherapy has been studied in detail.

5.2. Development of Resistance against existing antimalarials [132-134]:

Drug resistance in malarial parasite has become one of the most important problems in malaria control in recent years because of single drug therapy.[135, 136] Resistance *in vivo* has been reported in all antimalarial drugs, except artemisinin and its derivatives. Drug resistance necessitates the use of drugs which are more expensive and with dangerous side effects. In some parts of the world, artemisinin derived drugs are the first line of treatment, and are used indiscriminately for self treatment of suspected uncomplicated malaria, which ultimately will lead to resistance to artemisinin too, very soon. [137]

Drug resistance is common in Indo-Chinese penisula and the Amazon region of South America. The problem of drug resistance can be attributed primarily to increased selection pressures on *P. falciparum* in particular, due to indiscriminate and incomplete drug use for self treatment. In areas such as Thailand and Vietnam, mosquitoes of the *Anopheles dims* and *Anopheles minimus* species spread the drug resistant parasites. These mosquitoes adapt their biting activity to human behavior patterns, and maintain intense transmission. Drug resistant *P. falciparum* was first reported in Thailand in 1961. Various *P. falciparum* "strains" have now attained resistance to all commonly used and generally available antimalarial drugs. In man, the problem of resistance to the common antimalarial drugs, such as chloroquine and the decreasing effectiveness of quinine, is mainly limited to *P. falciparum* infection; chloroquine remains the treatment of choice for *P. vivax*.[138-139]

5.3. Combination therapy:

Since the efficacy of readily affordable antimalarial drugs is declining and the effective drugs are too expensive to common masses, cost-effective strategies are needed to extend the useful life spans of antimalarial drugs. The combination of two or more than twodrugs slows down the development of resistance and offers a synergistic effect too. However, most of the drug combinations are too costly to common masses. Malarone is a combination of proguanil and atovaquone and it has been established that both the drugs used together have a synergistic effect and this combination is now a very effective but costly antimalarial treatment. [140]

The drug combinations with or without artemisinin derivatives, including sulphadoxine combined with Mefloquine (MSP) and sulphadoxine combined with chloroquine, and many newer fixed combination drugs, having an additional artemisinin component, are being developed or marketed. Few of such

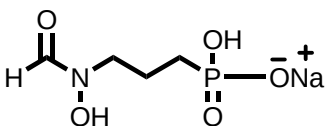
combination include chlorproguanil/dapsone (Lap Dap), [141] fansidar [142] and co-artemether (lumefantrine + artemether).[143] The effect of combination therapy is enhanced by the inclusion of an artemisinin derivative as artemisinin antimalarials decrease parasite density more rapidly than other antimalarial drugs However when used alone, the short half-life of the artemisinin derivatives minimizes the period of parasite exposure to sub therapeutic blood levels.

In combination with another drug having a longer half-life, the artemisinin derivatives with short half-life and rapid parasite clearance ensures that fewer parasites are exposed to the lone companion drug. Furthermore, exposure occurs when blood levels of the drug close to the maximum are still present. Another benefit of artemisinin combinations is the 90% reduction in gametocyte levels in treated patients. These characteristics minimize the probability that a resistant mutant will survive therapy and may also reduce overall malaria transmission rates.

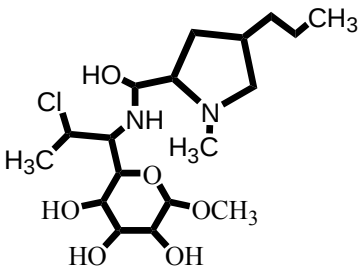
6. Recently reported novel compounds for Antimalarial chemotherapy:

Fosmidomycin:

Fosmidomycins belong to a class of antibiotics [144] the antimalarial activity of this class of molecules has been reported recently. The safety of the drug even in high doses (1.0 g every 6 h for 7 days) has been established.

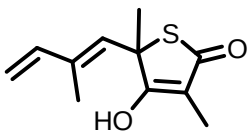


Clindamycin: It is a semisynthetic derivative of lincomycin and was introduced as in different formulations for the treatment of infections caused by *Plasmodium falciparum.* [145] Different studies have been carried out from time to time with clindamycin and derivatives alone as well as in combination with other compounds.[146]



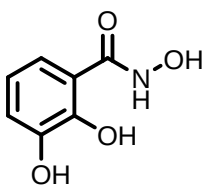
Clindamycin

Thiolactomycin: Thiolactomycin and many of its analogs have recently been shown to possess antimalarial activity, [89] and found to be the inhibitor of the Type-II fatty acid biosynthesis against DIVR (MDR w2mef) of *Plasmodium falciparum* .



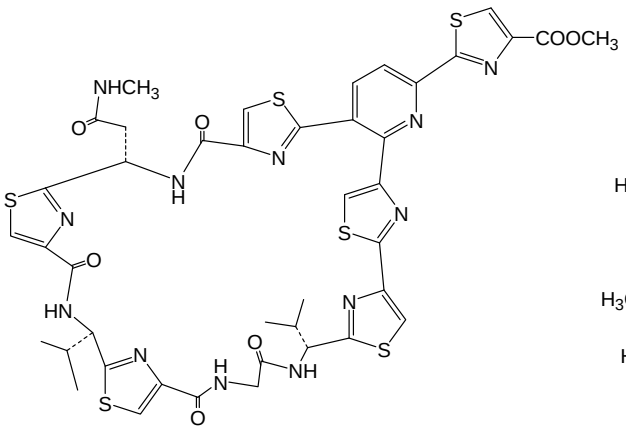
Thiolactomycin

Hydroxamates:[105,106,147] Some benzoyl hydroxamates have shown very good in vitro antimalarial activity**.** Very recently glycosyl hydroxamates were also found to be possessing antimalarial activities. The mode of action of these hydroxamates was found to be through chelation of iron and inhibition of peptide deformylase enzyme.

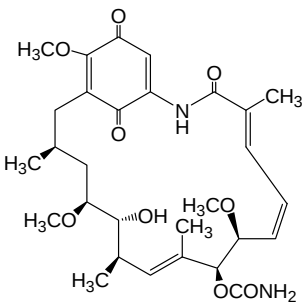


Thiopeptide antibiotics: [148a,b]

The thiopeptide antibiotics and in particular the amythiamicins, are a growing class of sulphur containing highly modified cyclic peptides characterised by several common



Amythiamicin D



Geldanamycin

structural features: the presence of thiazole and oxazole rings, unusual and dehydro amino acids, and a heterocyclic centrepiece of a tri- or tetra-substituted pyridine all in a macrocyclic array. Micrococcin is a potent growth inhibitor of the human malaria parasite *Plasmodium falciparum.* The thiopeptide antibiotics also have activity against the resistant strains of the malaria parasite *Plasmodium falciparum*,

7. Antimalarials from Natural /Plant Sources:

Most of the drugs currently available to treat malaria are quinoline derivatives modeled on the quinine molecule, found in the bark of *Cinchona* spp. trees found in high altitudes of South America.[149] In the XVI century, the plant bark and seeds were taken to Europe by the Jesuits and used for centuries to treat human malarial with efficacy. The quinine molecule from Cinchona inspired the synthesis of chloroquine and this drug became the chief replacement for quinine, during the Second World War. It is still widely used to treat malaria in areas where notable drug resistance has not yet appeared.

A number of natural products isolated from various plants have been reported to posses antimalarial activity. [150-153] Aqueous extracts of *Azadirachta indica* (bark), *Phyllanthus niruri* (whole plant) and *Ocimum sanctum* (leaves) were tested *in vitro* for their antimalarial activities using CQ sensitive isolate. IC_{50} values ranged from 0.3–70.0 µg ml-1.

8. Conclusion:

Malaria control is currently dependent on the effective, safe, affordable and practicable antimalarial drugs. The vector control without disturbing the ecosystem needs to be more strengthened with environment friendly biotechnological advancements. Unfortunately for several millions of malaria cases this disease is still economically less attractive for pharmaceutical companies and it is of scientific community to raise this issue to both the public and the Government agencies. Concerning the delayed developments of new antimalarial drugs, there is the need for a drug that allows short course treatment but not rapidly eliminated to delay the emergence of resistances. In order to delay the emergence of resistance and preserve effective drugs, combination therapy might be the solution. Artesunate derivatives are also found to be effective in reducing mortality of severe malaria and have no resistance besides the above aspects of manufacture, pricing, and making the prescribed regimen understandable, there must be surveillance for drug resistance and drug quality. The concerted effort of different governments towards new drug development programme against this disease would lead to an excellent R& D on Malaria.

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9. References:

1. Looareesu Walsh JA. Disease problems in the Third World. Ann N Y Acad Sci **1989**, *569*,1-16.
2. (a) Sachs, J.; Malaney, P. The conomic and social burden of malaria. *Nature* **2002**, *415,* 680-685.(b) Greenwood, B.; Mutabingwa, T. Malaria in 2002, *Nature* **2002**, *415*, 670-672 and references cited therein
3. Ridley, R. G. Medical need, scientific opportunity and the drive for antimalarial drugs. *Nature* **2002**, *415*,686-693.
4. **4.** Shell, E.R. Resurgence of a deadly disease. Atlantic Monthly, August **1997**.

5. Wirth, D.F. Biological revelations. *Nature* **2002**, *419,* 495-496.

6. R. Ross. *Studies on Malaria*, John Murray Publishers, London, **1928**.

7. Oaks SC, Mitchell VS, Pearson GW, Carpenter CCJ, editors. Malaria: obstacles and opportunities.Washington: National Academy Press; **1991**.

8. Foye, W. O. In *Principles of Medicinal Chemistry*; Foye, W. O., Lemke, T. L., Williams, D. A., eds.; Williams & Wilkins: Philadelphia, **1995**, *ch. 32*.

9. M. Balter, E. Marshall, G. Vogel, G. Taubes, E. Pennisi, M. Enserink. *Science* **2000**, *290*,428–441.

10. Lopez-Antinano, F. J.; Schmunis, G. A. Plasmodia of Humans. In Parasitic Protozoa; Academic: New York, **1993**, *5*,146–169.

11. Malaria: Obstacles and Opportunities; Oaks, S. C., Mitchel, V. S., Pearson, G. W., Carpenter, C. C. J., Eds.; *National Academy*: Washington, DC, **1991**.

12. Raynes, K. Bisquinoline antimalarials: their role in malaria chemotherapy *Int J.Parasitol*.**1999**,*29*,367-79 and references cited therein.

13. Snow, R.W, Omombo.J.A, Lowe. B., Molyneux, C.S., Obiero. J.O., Palmer. A. Heber. M.H., Pinder, m, Nahlem. B., Obonyo.C., Newbold, C, Gupta. S., Marsk. K. Relation between severe malaria morbidity in children and level of *Plasmodium falciparum* transmission in Africa. *Lancet* **1997**, *349*: 1650–1654.

14. Warrell, D.A. Molyneux., M.E, Beales. P.F.,. Severe and complicated malaria. *Trans.R. Soc. Trop. Med. Hyg*. **1990**, *84* ,1–65.

15. Wernsdorfer, W.H., and McGregor, I. Malaria: Principles and Practice of Malariology London: Churchill Livingstone, **1988**.

16. Bone, R.C., Toward a theory regarding the pathogenesis of the systemic inflammatory response syndrome: what we do and do not know about cytokine regulation. *Crit. Care Med.* **1996**, *24*:163–172.

17. Beeson, J.G., Brown, G.V., Pathogenesis of *Plasmodium falciparum* malaria: the roles of parasite adhesion and antigenic variation. *Cell Mol. Life Sci.* **2002**, *59*: 258–271.

18. Mons, B. Synchronised erythrocytic schizogony and gametogenesis of P. berghei in vivo and in vitro. *Parasitology* **1985**,*91*, 423-430,

19. Dondorp, A.M., Nyanoti, M., Kager, P.A., Mithwani, S., Vreeken, J., Marsh, K., The role of reduced red cell deformability in the pathogenesis of severe falciparum malaria and its restoration by blood transfusion. *Trans. R. Soc. Trop. Med. Hyg.* **2002**, *96*, 282–286.

20. Kroeger. A, Manecheno. M., Alareno. J. Pesse. K., Insecticide-impregnated bed-nets for malaria control: varying experiences from Ecuador, Colombia and Peru concerning acceptability and effectiveness. *Am. J. Trop. Med. Hyg.* **1995,** *53*, 313-323.

21. Lyimo, E. O., Msuya, F. H. M., Rwegoshora,R. T., Nicholson, E. A., Mnzava, A. E. P., Lines, J. D., . Curtis, C. F. Trial of pyrethroid-impregnated bed-nets in an area of Tanzaniaholoendemic for malaria. Part 3. Effects on the prevalence of malaria parasitaemia and fever. *Acta Trop.* **1991**, *49*, 157-163

22. Alonso, P L., Lindsay.S.W., Armstrong . J.R.M . Conteh, M., Hill, A.G., David , P.H., Fegan. G., Francisco.A.D., Hall . A.J. Shenton. F.C., Cham. K., Greenwood , B.M. The effect of insecticide treated bed-nets on mortality of Gambian children. *Lancet*, **1991**, *337*, 1499-1502

23. Mitchel C.J. Environmental management for vector control. In The Biology of disease Vectors. Eds. Beaty and W.C.Marquardt. University of Colorado, Colorado, P. 492, **1996**.

24. Vector control for malaria and mosquito borne diseases, report of a WHO study group, WHO Geneva, 1995.

25. Mitchel C.J., Environmental management for vector control in: The biology of disease vectors Edts B.J.Beaty and W.C.Marquardt University of Colorado, Colorado, 492, **1996**.

26. Mendis, K.N., Sina, B.J., Marchesini, P., Carter, R.,. The neglected burden of *Plasmodium vivax* malaria. *Am. J. Trop. Med. Hyg*. **2001,** *64*, 97–106.

27. Hisaeda, H., Stowers, A.W., Tsuboi, T., Collins, W.E., Sattabongkot, J.S., Suwanabun, N., Torii, M., Kaslow, D.C., Antibodies to malaria vaccine candidates Pvs25 and Pvs28 completely block the ability of *Plasmodium vivax* to infect mosquitoes. *Infect. Immun*. **2000**, *68*, 6618–6623.

28. Offman SL ASM Press, 1996, Malaria Vaccine Development: A Multi-immune Response Approach. Washington, DC

29. Migasena S, Heppner D. G, Kyle DE, Chongsuphajaisiddhi T, Gordon DM, Suntharasamai P, Permpanich B, Brockman A, Pitiuttutham P, Wongsrichanalai C, Srisuriya P, Phonrat B, Pavanand K, Viravan C, Ballou WR. SPf66 malaria vaccine is safe and immunogenic in malaria naive adults in Thailand. *Acta Trop.* **1997** , *67*, 215-27.

30. (a) Graves P, Gelband H. Review Cochrane Database Syst Rev. **2003**, (1):CD000129. (b)al-Yaman F, Genton B, Taraika J, Anders R, Alpers MP. Cellular immunity to merozoite surface protein 2 (FC27 and 3D7) in Papua New Guinean children. Temporal variation and relation to clinical and parasitological status. *Parasite Immunol*. **1997**, *19*, 207-14.

31. Tine JA, Lanar DE, Smith DM, Wellde BT, Schultheiss P, Ware LA, Kau€man EB, Wirtz RA, De Taisne C, Hui GS, Chang SP, Church P, Hollingdale MR, Kaslow DC, Hoffman S, Guito KP, Ballou WR, Sadoff JC, Paoletti E. NYVAC-Pf7: a poxvirus-vectored, multiantigen, multistage vaccine candidate for Plasmodium falciparum malaria. *Infect. Immun*. **1996**, 64,3833-44.

32. (a) Bojang KA, Milligan PJ, Pinder M, Vigneron L, Alloueche A, Kester KE, Ballou WR, Conway DJ, Reece WH, Gothard P, Yamuah L, Delchambre M, Voss G, Greenwood BM, Hill A, McAdam KP, Tornieporth N, Cohen JD, Doherty T; RTS, S Malaria Vaccine Trial Team. Efficacy of RTS,S/AS02 malaria vaccine against *Plasmodium falciparum* infection in semi-immune adult men in The Gambia: a randomised trial. *Lancet*, **2001**, *358*. 1927 - 1934. (b) Alonso PL, Sacarlal J, Aponte JJ, Leach A, Macete E,

Milman J, Mandomando I, Spiessens B, Guinovart C, Espasa M, Bassat Q, Aide P, Ofori-Anyinam O, Navia MM, Corachan S, Ceuppens M, Dubois MC, Demoitie MA, Dubovsky F, Menendez C, Tornieporth N, Ballou WR, Thompson R, Cohen J. Efficacy of the RTS,S/AS02A vaccine against Plasmodium falciparum infection and disease in young African children: randomised controlled trial. *Lancet*. **2004**, 16, 364, 1380-3.

33. Williamson K.C. Pfs230: from malaria transmission blocking vaccine candidate toward function *Parasite Immunol* **2003**, 25, 351-359 and references cited therein)

34. Tuteja, R. Critical Rev. Biochem. Mol. Biol. 2002, 37(1), 29-54 The development of a multivalent DNA vaccine for malaria.Hedstrom RC, Doolan DL, Wang R, Gardner MJ, Kumar A, Sedegah M, Gramzinski RA, Sacci JB Jr, Charoenvit Y, Weiss WR, Margalith M, Norman JA, Hobart P, Hoffman SL. Springer Semin *Immunopathol.* **1997**,*19*,147-59.

35. 35. (a) S. Cohen, I.A. McGregor, S. Carrington, Gamma-globulin and acquired immunity to human malaria, *Nature* **1961**,192, 733–737 (b) Hill, A. V., Allsopp, C. E., Kwiatkowski, D., Anstey, N. M., Twumasi, P., Rowe, P. A., Bennett, S., Brewster, D., McMichael, A. J. and Greenwood, B. M. **1991**.

36. (a) Common west African HLA antigens are associated with protection from severe malaria. *Nature* **352**, 595-600. (b) McConkey, S. J., Reece, W. H., Moorthy, V. S., Webster, D., Dunachie, S., Butcher, G., Vuola, J. M., Blanchard, T. J., Gothard, P., Watkins, K. et al. Enhanced T-cell immunogenicity of plasmid DNA vaccines boosted by recombinant modified vaccinia virus Ankara in humans. *Nat. Med.* **2003**. *9*, 729-735.

37. Sabchareon, A., Burnouf, T., Ouattara, D., Attanath, P., Bouharoun- Tayoun, H., Chantavanich, P., Foucault, C., Chongsuphajaisiddhi, T., Druilhe, P., Parasitologic and clinical human response to immunoglobulin administration in falciparum malaria. *Am. J. Trop. Med. Hyg*. **1991**, *45*, 297–308.

38. Cohen, S., McGregor, I.A., Carrington, S., Gamma-globulin and acquired immunity to human malaria. *Nature* **1961**,*192*, 733–737.

39. (a) L. Schofield, L. Vivas, F. Hackett, P. Gerold, R.T. Schwarz, S. Tachado, Neutralizing monoclonal antibodies to glycosylphosphatidylinositol, the dominant TNF-alpha-inducing toxin of *Plasmodium falciparum*: prospects for the immunotherapy of severe malaria, *Ann. Trop. Med. Parasitol* **1993,** *87*, 617–626. (b) Altman, J. D., Moss, P. A., Goulder, P. J., Barouch, D. H., McHeyzer- Williams, M. G., Bell, J. I., McMichael, A. J. and Davis, M. M. Phenotypic analysis of antigen-specific T lymphocytes. *Science* **1996,** *274*, 94- 96.

40. Herr, W., Schneider, J., Lohse, A. W., Meyer zum Buschenfelde, K. H. and Wolfel, T. Detection and quantification of blood-derived CD8+ T lymphocytes secreting tumor necrosis factor alpha in response to HLAA2.1-binding melanoma and viral peptide antigens. *J. Immunol. Methods* **1996**,*191*, 131-142.

41. Murali-Krishna, K., Altman, J. D., Suresh, M., Sourdive, D. J., Zajac, A. J., Miller, J. D., Slansky, J. and Ahmed, R. Counting antigenspecific CD8 T-cells: a reevaluation of bystander activation during viral infection. *Immunity* **1998**, *8*, 177-187.

42. De Jager, W., Te Velthuis, H., Prakken, B. J., Kuis, W. and Rijkers, G. T. (). Simultaneous detection of 15 human cytokines in a single sample of stimulated peripheral blood mononuclear cells. *Clin. Diagnost. Lab. Immunol.* **2003**, *10*, 133-139.

43. The Lancet editorial, Malaria after the genomes. *The Lancet* **2002,** 360, 1107**.**

44. Horrocks, P., Bowman, S., Kyes, S., Waters, A. & Craig, A. Entering the post-genomic era of malaria research. *Bull. World Health Organ.* **2000,** *78,* 1424–1437.

45. Buysse, J. The role of genomics in antibacterial target discovery. *Curr. Med. Chem.* **2001,** *8,* 1763–1776 .

46. Gardner MJ, Hall N, Fung E, White O, Berriman M, Hyman RW, Carlton JM, Pain A, Nelson KE, Bowman S, Paulsen IT, James K, Eisen JA, Rutherford K, Salzberg SL, Craig A, Kyes S, Chan MS, Nene V, Shallom SJ, Suh B, Peterson J, Angiuoli S, Pertea M, Allen J, Selengut J, Haft D, Mather MW, Vaidya AB, Martin DM, Fairlamb AH, Fraunholz MJ, Roos DS, Ralph SA, McFadden GI, Cummings LM, Subramanian GM, Mungall C, Venter JC, Carucci DJ, Hoffman SL, Newbold C, Davis RW, Fraser CM, Barrell B.Genome sequence of the human malaria parasite Plasmodium falciparum Nature. **2002,***419*, 498-511.

47. White J. J. N. Current concepts; in treatment of malaria. *Engl J Med*, **1996**, *335*, 800-806.

48. Luzzi G.A., Peto T.E.A., Treatment of malaria *Drug Saf*, **1993**, *8*, 295-311.

49. Keystone, J.S. Quinine-induced cutaneous vasculitis**.** *Drugs,* **1990**, *39*, 337-354.

50. Meinnel, T. Peptide deformylase of eukaryotic protists: a target for new antiparasitic agents? *Parasitology Today,* **2000**, *16* 165-68.

51. Ridley Robert C. Medical need, Scientific Opportunity and the drive for antimalarial drugs *Nature* **2002**, *415*, 686-693.

52. Report no WHO/CDS/RBM/2001.33 (World Health Organization, Geneva 2001.)

53. Report no WHO/CDS/RBM/2001.35 (World Health Organization, Geneva 2001.

54. Report no WHO/MAL/98.1086 (World Health Organization, Geneva 1998.)

55. Winstanley, P. Chemotherapeutic options for malaria *Lancet*, **1996**, *348*, 1196-1201.

56. BannisteR, L. A brief guide to ultrastructure of P. falciparum asexual blood stages *Parasitol. Today* **2000**, *16*, 427-433.

57. Fry, M. Beesley, J. Mitochondria of mammalian Plasmodium spp. *Parasitol. Today* **1999**, *102*, 17-26.

58. Krungkrai J, Krungkrai SR, Suraveratum N, Prapunwattana P. Mitochondrial uniquinol-cytochrome c reductase and cytochrome c oxidase: chemotherapeutic targets in malarial parasites. *Biochem Mol Biol Int.* **1997**, *42*,007-14.

59. Vaidya, A. in Antimalarial Chemotherapy, Mechanism of action, Resistance and new directions in Drug Discovery (ed. Rosenthal P.) Totawa, N J, Humana Press **2001**, PP 203-218.

60. Alzeer. I., Collet. J., hemze., Krauss., hebschwerbn, C., matile. H., Ridley. R.G., Phenyl β-methoxy acrylates Alzeer, I. et.al. Phenyl β-methoxy acrylates: a new antimalarial pharmacophore *J. Med. Chem*. **2000**, *43*, 560-568.

61. (a) Woodrow,C.J., Penny J.I. and Krishna, S. Intraerythrocytic *Plasmodium falciparum* expresses a high affinity facilitative hexose transporter. *J. Biol. Chem*. **1999**, *274,* 7272-7277 and refrences cited therein (b) Fry, M.; Webb, E., P udney, M. *Comp. Biochem. Physiolo B*, **1990**,*96*, 775-782

62. Roth, E. Rosa, R. The use of enzymopathic human red cells in study of malarial parasite glucose metabolism. *Blood* **1988**,*71*,1408–1413 and references cited therein

63. Brady. R.L., Structure based approach to the development of novel antimalarials *Current Drugs* **2004**, *5,* 137-149.

64. Vander, J.D.L, Hansdkn. L.A., Heidrech J E., Partial participation and of lactic dehydrogenate *Plasmodium falciparam Mol*. *Biochem. Parasitol*. **1981**, *4*, 255-264.

65. Rastelli, G., Sirawaraporn, W.,Sompornpisut, P., Vilaivan, Kamchonwongpaisan, S., Quarrel, R.,Lowe, G., Thebtaranonth, Y., Yuthavong, Y., Interaction of pyremethamine ,cycloguanil, WR99210, and

their analogs with *P. falciparum* dihydrofolatereductase: structure basis of antifolate reductase *Bioorg. Med. Chem.* **2000**, *8*, 1117.

66. Clough, B. and Wilson, R. J. M. Antibiotics and the plasmodial plastic organelle. In Antimalarial Chemotherapy (ed. P.J. Rosenthal), T Totawa, N J, Humana Press otawa, N J, Humana Press.**2001**, pp. 287-312.

67. Canfield, C.J. Pudney M. and Gutteridge, W. E. Adverse effects of antimalarials. An update. *Exp. Parasitol* **1995,** *80*, 505-516

68. Francis, S.; Sullivam, D.J. and Goldberg, D. Haemoglobin metabolism *Annu. Rev. Microbiol*. **1997**, *51*, 97-123.

69. Combs, G. H., Golberg, D.E., Klemba, M., Berry. C., Kay, J., Mottram, J.C. Aspartic proteases of P. falciparum and other parasite protozoa as drug targets *Trends in Parasitol*. **2001**, *17*, 532-537

70. Shenai B., Sijwali, P., Singh A., Rosenthal P. Characterization of native and recombinant falciparin2 a principal trophozoit Cystein proteinase and essential hemoglobinase of *P. falciparum J. Biol. Chem*. **2000**, *275*, 29000-29010

71. Eggelson, K., Duttin, K.; and Gloldberg, D. Identification and Characterization of falcilysin, metallopeptidase involved in haemoglobin catabolism with malarial parasite P. falciparum *J. Biol. Chem*.**1999**, *274*, 32411-32417

72. McKerrow J. H, Sun E, Rosenthal P. J, Bouvier J. The proteases and pathogenicity of parasitic protozoa *Annu Rev Microbiol* **1993**. *47*, 821-53.

73. Noteberg D, Hamelink E, Hulten J, Wahlgren M, Vrang L, Samuelsson B, Hallberg A. Design and synthesis of plasmepsin I and plasmepsin II inhibitors with activity in Plasmodium falciparum-infected cultured human erythrocytes. *J Med Chem*. **2003,** *46*, 734-46.

74. Silva AM, Lee AY, Gulnik SV, Maier P, Collins H, Bhat TN, Collins PJ, Cachau RE, Luker KE, Gluzman IY, Francis SE, Oksman A, Goldberg DE, Erickson JW. Structure and inhibition of plasmepsin II, a hemoglobin-

degrading enzyme from *Plasmodium falciparum. Proc Natl Acad Sci USA.* **1996**, *93*, 10034-9.

75. Rosenthal, P. J, Meshnick S. R. Hemoglobin catabolism and iron utilization by malarial parasite *Mol Biochem Parasitol* **1996**, *83*, 131-9.

76. Slater, A. F.G. Malaria Pigment *Exp Parasitol* **1992**, *74*, 362-5.

77. Bond, J.S., Butler, P.E. Intracellular Proteases *Annu Rev Biochem* **1987**, *56*, 333-64.

78. Gluzman, I. Y., Francis, S.E., Oksman, A., Smith, C.E., Duffin, K.L., Goldberg D.E. Order and specificity of the Plasmodium falciparum hemoglobin dgradation pathway. *J Clin Invest* **1994**, *93*, 1602-8.

79. Chow, A.; Cherti, R., Fitch, C. Ferriprotoporphyrin IX fulfills the criteria for identification as chloroquin receptor of malaria parasite *Biochemistry,* **1980**, *19*, 1543-1549.

80. Sullivan, D.J., Gluzerman, I., Russel, D., Goldberg, D. On the Molecular Mechanism of Chloroquin's antimalarial action *Proc. Natl. Acad. Sciences* **1996**, *93*, 11865-11870.

81. Bray, P., Mungthin, M., Ridley, R., Ward, S., Access to hematin: the basis of chloroquin resistance. *Mol. Pharmacol*. **1998**, *54*, 170-179.

82. Mungthin, M., Bray, P., Ridley, R., Ward, S., Central role of hemoglobin degradationin mechanism of action of 4-amino quinolines, quinoline methanols and phenanthrene methanols, *Antimicrob. Agents. Chemother*. **1998**, *42*, 2973-2977.

83. Sullivan, D.J., Matile, H., Ridlley, R., Goldberg, D. A common mechanism for blockade of heme polymerization by antimalarial quinolines *J. Biol. Chem.*. **1998**, 273, 31103-31107.

84. Meshnik, S., Talor, T., Komchonwongpaison, S.; Artemesnin and antimalarial endoperoxides: from herbal remedy to targeted chemotherapy. *Microbiol. Rev*. **1996**, *60*, 301-315.

85. Meshnik, S., in Antimalarial chemotherapy, Mechanism of Action, Resistance and new directions in drug discovery (ed. Rosenthal P) 191-202 (Humana, Totowa, New Jersy, 2001)

86. Foth, B. J., Ralph, S. A., Tonkin, C. J., Struck, N. S., Fraunholz, M., Roos, D. S., Cowman, A. F. and McFadden, G. I. Dissecting apicoplast targeting in the malaria parasite *Plasmodium falciparum*. *Science* **2003**, *.299*, 705-708.

87. Ralph, S. A., D'Ombrain, M. C. and McFadden, G. I. The apicoplast as an antimalarial drug target. *Drug Resist. Update* **2001**, *4*, 145-151.

88. Roos, D. S., Crawford, M. J., Donald, R. G., Fraunholz, M., Harb, O. S., He, C. Y., Kissinger, J. C., Shaw, M. K. and Striepen, B. Mining the Plasmodium genome database to define organellar function: what does the apicoplast do? *Philos. Trans. R. Soc. Lond. B. Biol. Sci.* **2002**, *357*, 35-46.

89. Jones, S.M., Urch, J.E., Brun, R., Harwood, J.L., Berry, C., Gilbert, I.H. Analogs of Thiolactomycin as potential antimalarial and antitrypnosomal agents *Biorganic Med Chem*. **2004**, *12*, 683-692 and refrences cited therein.

90. Surolia, N., Surolia, A., Triclosan offers protection against blood stages of malaria by inhibiting enoyl-ACP-reductase of Plasmodium falciparum *Nature Med*. **2001**, *7*, 167-173.

91. Jornna H., Wiesner, J., Sandarbrand, S., Altincicek,B., Weidemeyer, C., Hintz, N., Turvaehova, I., Eberal, M., Zeidler, J. Lichtenthaler, H.K., Soldati, D.; Beck., E. Inhibition of nonmevalonate pathway of isoprenoid biosynthesis as antimalarial drug *Science* **1999**, *285*, 1573-1576.

92. Wiesner J, Borrmann S, Jomaa H. Fosidomycin for the treatment of malaria. *Parasitol Res*. **2003,** June: 90 Suppl. 2:S71-76

93. Gerold, P., Dieckmann-Schuppert, A., Schwarz, R.T., Glycosylphosphatidylinositols synthesized by asexual erythrocytic stages of the malarial parasite, *Plasmodium falciparum.* Candidates for plasmodial glycosylphosphatidylinositol membrane anchor precursors and pathogenicity factors, *J. Biol. Chem* **1994,** *269*, 2597–2606.

94. Gowda, D.C., Gupta, P., Davidson, E.A. Glycosylphosphatidylinositol anchors represent the major carbohydrate modification in proteins of intraerythrocytic stage *Plasmodium falciparum*, *J. Biol. Chem* **1997**, *272*, 6428–6439.

95. Eckert, V., Gerold, P., Schwarz, R.T. GPI-anchors: structure and functions, in: H.J. Gabius, S. Gabius (Eds.), Glycosciences, Chapman & Hall,Weinheim,, Germany, **1997**, pp. 223–243.

96. Schofield, L., Tachado, S.D., Regulation of host cell function by glycosylphosphatidylinositols of the parasitic protozoa, *Immunol. Cell Biol* **1996**, *74*, 555–563.

97. Gerold P., Jung, N. Azzouz, N. Frieberg, N., Kobe, S.; Schwarz, R.T. biosynthesis of glycosylphosphatidylinositols of *Plasmodium falciparum* in a cell free incubation system: inositol acylation is needed for in annosylation of glycosylphosphatidylinositols *Biochem. J.* **1999**, *344*, 731-738 and references cited therein.

98. Vial, H.J. Thuet, M.J., Broussal, J.L, Philippot, J.R. Phospholipid biosynthesis by *Plasmodium knowlesi*-infected erythrocytes: the incorporation of phospholipids precursors and the identification of previously undetected metabolic pathways, *J. Parasitol* **1982**, *68,* 379–391.

99. Hsiao, L.L., Howard, R.J., Aikawa, M., Taraschi, T.F., Modification of host cell membrane lipid composition by the intra-erythrocytic human malaria parasite *Plasmodium falciparum*, *Biochem. J.* **1991**, *274* 121–132.

100. Mitamura T. and Palacpac, N.M.Q. Lipid metabolism in *Plasmodium falciparum*-infected erythrocytes: possible new targets for chemotherapy Microbes and Infection **2003,** *5*, 545-552.

101. Matesanz, F., Duran-Chica, I. and Alcina, A. The cloning and expression of *Pfacs1*, a *Plasmodium falciparum* fatty acyl coenzyme a synthetase-1 targeted to the host erythrocyte cytoplasm. *J. Mol. Biol.* **1999**, *291*, 59-70.

102. Meinnel T. Methionine as translational start signal: A review of the enzymes of the pathway in *E. coli*. *Biochimie*, **1993**, *75*, 1061-75

103. Meinnel T. Peptide deformylase of eukaryotic protists: A target for new antiparasitic agents. Parasiology Today, **2001**, 16(4), 165-68.

104. Rogers, M.J., Cundliffe, E., Mc Cutehan, T.F. The antibiotic microccine is a potent inhibitor of growth and protein synthesis in the malarial parasite *Antimicrob. Agents. Chemother*. **1998**, *42*, 715-716.

105. Meinnel, T., Patiny, N., Ragusa, S., Blanquet, S. Design and synthesis of substarte analogue inhibitors of peptide deformylase *Biochemistry* **1999**, *38*, 4287-4295.

106. Mishra,R.C., Tripathi, Renu, Katiyar, D., Tewari, N., Singh, D. and Tripathi, R.P Synthesis of Glycosylated β-Amino Hydroxamates As New Class of Antimalarials,. *Bio. Med. Chem*., **2003**, *11*, 5363-5374.

107. Scheibel, L. W., Adler, A. and Trager, W. Tetraethylthiuram disulfide (Antabuse) inhibits the human malaria parasite *Plasmodium falciparum. Proc. Natl. Acad. Sci. USA* **1979**, *76*, 5303–5307

108. Becker, K., Rahlfs, S., Nickel, C., Schirmer, R.H., a. Glutathione-function and metabolism in the malarial parasite *Plasmodium falciparum*. *Biol. Chem.* **2003**, *348*, 551–566.

109. Rainer M. Luond, James h.Mckie, Kenneth T.Douglas, Micheal J.Dascombe and Janet vale, Inhibitors of glutathione reductase as potential antimalarial drugs . Kinetic cooperativity and effect of Dimethyl Sulphoxides on inhibition Kinetics *J.Enzyme inhibition* **1998,** *13*,327-345.

110. 110. Panisko D M, Keystone J S. Treatment of malaria *Drug.* **1990**, *39*, 160-189..

111. Price, E.J, Bevan JS, Rees A Quinine –induced cutaneous vasculitis.*Br. J. Clin. Pract* .**1992,** *36*, 138-139

112. Gottschall, J.L., Elliot, W., Lianos, E. Farland, J.G.M., Biolfmeyer, K., Aster, R.H. Quinine –induced immunthrombocytopenia associated with hemolytic uremic syndrome-a new clinical entity *Blood* **1991**, *77*, 306-310.

113. Glynne, P. , . Quinine –induced immunthrombocytopenia purpura followed by hemolytic uremic syndrome *Am. J. Kidney Dis*. **1999**, *33*, 1333-137.

114. Dollery, C., ed. Chloroquin, in therapeutic drugs (2nd edition)pp C177-C182, Churchil Livingston, **1999**.

115. 115. Wernsdorfer, W. H. The development and spread of drug-resistant malaria. *Parasitol. Today*, **1991**. ***7***, 297-303 and references cited therein.

116. Bruce-Chwatt, L.J. Black R.H. Canfield, C.J. Clyde, D.F. Peters, W., Wernsdorfer, W.H., (Eds.) Chemotherapy of Malaria 2nd edition, WHO; Geneva **1986.**

117. Dollery, C., ed. Chloroquin, in therapeutic drugs (2nd edition 1999) Mefloquin pp M24-M30, Churchil Livingston.

118. Winstanley, P.A. Mefloquin: the benefits outweigh the risks Br. *J. Clin. Pharmacol*. **1996**, *42*, 411-414.

119. Dollery, C., ed. Chloroquin, in therapeutic drugs (2nd edition 1999) Halofrantin, H1-H2 Churchil Livingston.

120. Milton, K., Edward , G., Hard , S.A., orme, M.L., And Breckenridge., A. M., The effect of food on the pharmacokinetics of of Halofantrine in man after the administration of single oral dose *Br. J. Clin. Pharmacol*. **1989**, *27*, 671-672.

121. Monlun E, Leenhardt A, Pillet O, Gaston R, Receveur MC, Bouabdallah K, Longy-Boursier M, Favarel-Garrigues JC, Le Bras M...

Ventricular arrhythmias after malaria cure by halofantrin-three cases *Bull. Soc. Path. Exot.* **1993**, *86*, 365-368.

122. Puri SK, Singh N. Azithromycin: antimalarial profile against blood and sporozoite-induced infections in mice and monkeys. *Exp Parasitol*. **2000**, *94,* 8-14.

123. Winstanley, P. A. Chemotherapy for falciparum malaria: the armoury, the problems and the prospects. *Parasitol. Today* **2000**,*16*,146-153

124. De Vries PJ, Dien TK. Clinical pharmacology and therapeutic potential of artemisinin and its derivatives in the treatment of malaria. *Drugs* **1996**,*52*, 818-36.

125. Anon, T. Use of artemesinin and its derivatives as antimalarial drugs. Report of a joint CTD/DMP/TDR informal consultation. Malaria Unit,Division of control of tropical diseases WHO **1995.**

126. Paktiya Teja-Isavadharm, James O. Peggins, Thomas G. Brewer, Nicholas J. White, H. Kyle Webster, and Dennis E. Kyle *Plasmodium falciparum*-Based Bioassay for Measurement of Artemisinin Derivatives in Plasma or Serum Antimicrob Agents Chemother**. 2004** ,48, 954–960.

127. Van Hensbroek M.B.Tran Tinh Hien ., Nicholas, P.J. Day. Nguyen Hoen Nguyen Thi Hoang Mai., Tran Thi Hong Chau , Dinhxuan sinh ., Ly Van., Chuong, Ha vinh, Deborah, Waller, B.M., B., Chir., Timonthy. E. A,Peto, D Phil., B.M., and Nicholas. J.White, A controlled trial of aremether or quinine in children with cerebral malaria *New. Engl. J. Med.* **1996,** *335,* 76-83.

128. Hieh ,T. A control lled trial of artemether or quinine in children with cerebral malaria . New Engl. J., Med.**1996**, *335* ,76-83.

129. Murphy , S., English. M, Waruiru,cx., Mwangi, I., Amukoye. E., Crawley, J., Newton, C., Winstanley, P.,Peshu,N., and Marsk.K., An

open randomised trial of artemether versus quinine in the treatment of cerebral malaria in african children .*Trans .R . Soc. Trop.Med. hyg.* **1996**,*90*, 298-301.

130. Pittler, M.H. and Ernst, E. Artemether for severe malaria : a meta analysis of randomised clinicals trials . *Clin. Infect. Dis.* **1999**, *28*, 597-601.

131. Murphy , S.A., Mberu, E., Mahia, D., English. M., Crawley. J., Waruiru, C., Lowe. B., Newton. C.r. J., HInstanley., P., Marsh, K., and Watkins. W.M., The disposition of intramascular artemether in children with cerebral malaria : a prelimnary study . *Trans . R. Soc . Trop. Med. Hyg*. **1997** , *91*, 331-334.

132. Foote, S. J., and A. F. Cowman. The mode of action and the mechanism of resistance to antimalarial drugs. Acta Trop. **1994**, *56***,** 157-171

133. Peters, W. 1987 Chemotherapy and drug resistance in malaria, 2nd ed. Academic Press, London, United Kingdom

134. Pudney, M. Antimalarials: from quinine to atovaquone. *Soc. Gen. Microbiol* **1994**, *53*, 229-247.

135. Bunnag D., Viravan C., Looareesuwan S., *et al.* Clinical trial of artesunate and artmether in multi-drug resistant falciparum malaria in Thailand: a preliminary report. *Southeast Asian J. Trop. Med. Public Health***. 1991**, *22*, 380-385.

136. Murphy GS, Basri H, Purnomo, Andersen EM, Bangs MJ, Mount DL, Gorden J, Lal AA, Purwokusumo AR, Harjosuwarno S**,** Vivax malaria resistant to treatment and prophylaxis with chloroquine. . *Lancet* **1993,** *341*, 96–100.

137. Price, R. N., F. Nosten, C. Luxemburger, M. van Vugt, L. Phaipun, T. Chongsuphajaisiddhi, and N. J. White**.**. Artesunate/mefloquine treatment of multi-drug resistant falciparum malaria. *Trans. R. Soc. Trop. Med. Hyg.* **1997**, *91***,** 574-577.

138. White, N. J. Antimalarial drug resistance: the pace quickens. *J. Antimicrob. Chemother*. **1992**, *30*, S71-S78

139. Schuurkamp, G. J., P. E. Spicer, R. K. Keren, P. K. Bulungol, and K. H. Rieckmann**.** Chloroquine-resistant *Plasmodium vivax* in Papua New Guinea. *Trans. R. Soc. Trop. Med. Hyg*. **1992**, *86,*121-122

140. . Bloland PB, Kazembe PN, Watkins WM, Doumbo OK, Nwanyanwu OC, Ruebush TK 2nd. Malarone-donation programme in Africa *Lancet*, **1997**, *350*, 1624-1625 and references cited therein

141. Winstanley. P, Watkins. W, Muhia. D, Szwandt. S , Amukoye. E, Marsh. K, Chlorproguanil/dapsone for uncomplicated *Plasmodium falciparum* malaria in young children: pharmacokinetics and therapeutic range *Transactions of the Royal Society of Tropical Medicine and Hygiene,* **1997,** *91,322-327*

142. (a) Salako, L.A,. Adio. R.A, Sowunmi.A, and Walker.O, Parenteral sulphadoxine-pyrimethamine (Fansidar): an effective and safe but under-used method of anti-malarial treatment *Transactions of the Royal Society of Tropical Medicine and Hygiene* **1990**, *84*, 641-643. (b) Hellgren U, Kihamia CM, Bergqvist Y, Lebbad M, Premji Z, Rombo L. Standard and reduced doses of sulfadoxine-pyrimethamine for treatment of *Plasmodium falciparum* in Tanzania, with determination of drug concentrations and susceptibility *in vitro*. *Trans R Soc Trop Med Hyg* **1990**,*84*, 469-73

143. Toovey, A. Jamieson and G. Nettleton **.**Successful co-artemether (artemether–lumefantrine) clearance of falciparum malaria in a patient with severe cholera in Mozambique *Travel Medicine and Infectious Disease,* **2003***, 1,* 177-179.

144. Kuemmerle, H. P., T. Murakawa, K. Soneoka, and T. Konishi.. Fosmidomycin: a new phosphonic acid antibiotic. Part I: phase I tolerance studies. *Int. J. Clin. Pharmacol. Ther. Toxicol.* **1995,** *23*,515–520.

145. Clyde,D.F., Gilman, R. H., McCarthy, Antimalarial effects of Clindamycin in man *Am. J. Trop.Med.Hyg* **1975**, *24.* 3659-370.

146. Lell, B., Kremsner, P.G., Clindamycin as antimalarial drug :Review of clinical trials Antimicrobial agents and chemotherapy **2002**, *476*, 2315-20 and references cited therein.

147. Hynes JB, Hack LG. Hydroxylamine derivatives as potential antimalarial agents. 2. Hydroxamates and amidoximes. *J Med Chem*. **1972**,*15*, 194-6.

148. (a) Bagley, A. M. C., Bashford, K. E., Hesketh, C. L., Moody, C. J. Total Synthesis of thiopeptide promothiocin *J. Am. Chem. Soc.*, **2000**, *122*, 3301-3313. (b) Moody, C. J., Bagley, M. C. Total synthesis of nostocyclamide. *J. Chem. Soc., Perkin Trans. 1*, **1998**, 601-607.

149. Garnham, P.C.C., Malaria Parasites and other heamosporidia, Blackwell Scientific Publications, Oxford, 1114pp and references cited therein.

150. Bilia. A.R, Lazari. D, Messori. L, Taglioli .V, Temperini. C, Vincieri FF. Simple and rapid physico-chemical methods to examine action of antimalarial drugs with hemin: its application to *Artemisia annua* constituents.*Life Sci*. **2002**, *7,*769-78.

151. Francois. G, Timperman. G, Steenackers. T, Assi L.A, Holenz. J, Bringmann. G. In vitro inhibition of liver forms of the rodent malaria parasite Plasmodium berghei by naphthylisoquinoline alkaloids--structure-activity relationships of dioncophyllines A and C and ancistrocladine.*Parasitol Res*. **1997**, *83,* 673-9.

152. Leaman D.J, Arnason J.T, Yusuf .R, Sangat-Roemantyo. H, Soedjito. H, Angerhofer .C.K, Pezzuto J.M. Malaria remedies of the Kenyah of the Apo Kayan, East Kalimantan, Indonesian Borneo: a quantitative assessment of local consensus as an indicator of biological efficacy.*J Ethnopharmacol*. **1995** *49*(*1*), 1-16.

153. *Phillipson J D.* Natural products as drugs.*Trans R Soc Trop Med Hyg*. **1994**,*88*,17-9.