Antimalarial Drug Development: Past to Present Scenario

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Abstract: Malaria is a global health problem that needs attention of drug discovery scientists to investigate novel compounds with high drug efficacy, safety and low cost to counter the malaria parasites that are resistant to existing drug molecules. This is an overview of past to present status of antimalarial drugs including newly researched candidates and also the alternative approaches for the complete control of malaria.

Key Words: Malaria, antimalarials, drug-resistance, combination therapy, vaccines.

1. INTRODUCTION

Malaria is endemic in large parts of the world, particularly in the tropical and subtropical regions. According to WHO there were 247 million malaria cases and more than one million people, mostly children less than 5 years in age die each year. 109 countries were endemic for malaria in 2008, amongst them 45 are in the African region [1]. The WHO defines malaria as an infectious disease caused by protozoa of the genus *Plasmodium*; episodes of illness are "attack of chills, fever and sweating". Four different Plasmodium species infect humans and cause distinct disease patterns: *P.falciparum* (malaria tropicana), *P. vivax* (malaria tertiana), *P. malariae* (malaria tertiana), and *P. ovale* (malaria quartana). Out of these four species, *P. falciparum* is the most dangerous form of malaria [2]. The parasite, *Plasmodium*, was found to have a life cycle split between the anopheles mosquito vector and the human host, (Fig. (**1**)) [3].

In the recent years many compounds have been discovered and implemented in the treatment of malaria. The design of drug molecules was targeted on different stages of life cycle of the malaria parasite (Fig. **1**). In various categories a number of leading drug molecules have proven great success for the treatment of malaria from past to present. The first attempt for specific treatment of malaria dates back to the early 18th century and made use of the bark of cinchona trees. Quinine was the first drug to be used for the treatment of malaria in 1820s. Since then quinine and its derivatives such as pamaquine, chloroquine, primaquine and amodiaquine etc. dominated the landscape of malaria treatment for a century. In the 1930s other synthetic agents like sulfadoxine, proguanil and cycloguanil were added to the pipeline and widened the choice. In between the phenomenon of resistance skyrocketed against all the established drugs and limited their use. Two decades later endoperoxide artemisinin and its derivatives supplanted the quinolines and other drugs. They provided the benchmark for the rational design of many promising trioxanes and endoperoxide antimalarials with no resistant liabilities. Various natural compounds are being recently exploited for possible source of new lead. As infectious diseases evolve and develop resistance to existing drugs, many compounds generate a space for further improvement and development of new molecules against malaria viz. double drug approaches and metal complexes of quinolines.

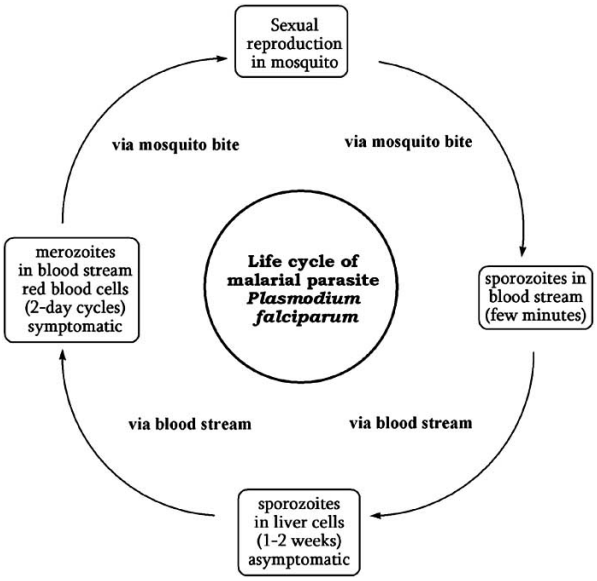

Fig. (1). Life cycle of malaria parasite.

Traditional attempts for the treatment of malaria with conventional chemotherapeutic methods are failing because of the development of resistant strains. Antimalarial drug resistance develops when spontaneously occurring parasite mutants with reduced susceptibility are selected, and are then transmitted [4, 5]. Resistance has emerged to most of the drugs except the artemisinins and its derivatives and this is responsible for a recent increase in malaria-related mortality, particularly in Africa [6].

Presently most of the research is focused on the development of new drug molecules, eradication of mosquitoes and development of an effective vaccine. Despite major re-

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search activities and the complete genome sequencing of the malaria parasite, one of the major bottlenecks in the development of an effective vaccine is complicated by the fact that different antigens are expressed in different stages of the parasite, and a vaccine efficient in killing liver-stage parasites may not inhibit the growth of blood-stage parasites [7].

As vaccine development has also not met with success, currently multidrug combinations offer temporary relief [8]. Combinations potentially offer a number of important advantages over monotherapies. Ideally, combination regimens will incorporate two agents that are both new, offer potent synergistic activity and preferably have similar pharmacokinetic profiles. In combination with new drugs and formulations some of which will arise from genome sequencing projects, malaria will hopefully soon come under complete control [9]. Slight modifications of existing drugs or the design of novel agents that act against new targets are the basic strategies that are followed in the development of new antimalarials [10]. New investigational drugs that are being developed and tested for efficacy in subsequent clinical trials now have to go through new phase III clinical trial designs, whereby they are needed to be compared against current ACTs (Artemisinin-based Combination Treatment) based regimens of high efficacy [11].

This review intends to highlight the currently available drugs including various novel agents. In addition, emphasis has been given on the prospective pharmacophores that are likely to emerge as effective clinical candidates in the treatment of malaria. Besides some alternative approaches are also highlighted.

2. CHEMOTHERAPY OF MALARIA

Antimalarial drugs are designed to prevent or cure malaria. Two types of antimalarial drugs are to be distinguished: one taken as preventive called prophylactic drugs, and those which are taken once the person is already infected, called therapeutic drugs. Chemically antimalarial compounds are broadly classified into two categories Table (**3**).

2.1. Nitrogen Containing Heterocyclic Compounds

a) 4-Aminoquinolines and Aryl Aminoquinolines

4-aminoquinolines and aryl amino-alcohols are structurally derived from quinine (**1**), the active ingredient of the cinchona bark. Despite its relatively low efficacy and tolerability, quinine still plays an important role in the treatment of multi resistant malaria. The mean elimination half life of quinine is between 10 to 12 hours [12]. It has schizontocidal effect against all plasmodium species infecting man. It inhibits heme polymerization and heme catalyzed activity in malaria parasite within food vacuole but with less extent than chloroquine [13]. Chloroquine (**2**) is a 4-aminoquinoline anti-malarial and a very potent blood schizonticidal drug. It was synthesized in 1934, but was not used until 1946 because it was considered to be too toxic [14]. It is very cheap and very effective against the erythrocytic forms of all four plasmodial species, but not against the liver forms. Lysomotropic character of chloroquine is believed to account for much of its antimalarial activity [15,16]. The drug concentrates in the acidic food vacuole of the parasite. It also inhibits heme polymerase, the enzyme that polymerizes heme to hemozoin [17]. In an attempt at widespread distribution of the drug as a prophylactic, chloroquine was added to table salt in parts of South America, Africa, and Asia. At the end of 1980s first case of resistance appeared and it was thought to be because of the salt programme [18]. Adverse reactions commonly associated with chloroquine include severe gastritis, difficulty in accommodation, blurring of vision, corneal opacity, toxic psychosis, photosensitive dermatoses and even retinal damage on prolonged use [19].

The development of chloroquine derivatives such as bisquinolines viz. metaquine (**3**) in which two quinoline cores are connected by various linkers is the focus of work on heme-polymerization inhibitors [20, 21]. In addition to heme-polymerization inhibitors, inhibitors of fatty acid synthesis, choline uptake inhibitors, farnesyl transferase inhibitors, and glyceraldehyde-3-phosphate dehydrogenase inhibitors are under preclinical development [22].

Mefloquine (**4**) and halofantrine (**5**) are aryl amino alcohols that are very similar to quinine [23, 24]. Halofantrine was developed in the 1960s by the Walter Reed Army Institute of Research and the same institute developed mefloquine in 1970s. It is believed that both share similar mechanism of action. Halofantrine is used in the treatment of chloroquine resistant and multi-drug resistant, uncomplicated *P. falciparum* malaria. Halofantrine exhibits low bioavailability by the oral route and has a potential arrhythmogenic effect, leading to a prolongation of the QT interval in the electrocardiogram (ECG) [25-29]. Halofantrine, although highly effective but it is found to have cross-resistance with mefloquine and induces cardiac arrhythmia [30]. Mefloquine, a synthetic analogue of quinine was used during the Vietnam War for chloroquine resistant *P. falciparum* malaria. It is generally well tolerated in therapeutic doses but subsequent appearance of harmful neuropsychiatric effect and a high cost limit its use [31, 32].

The 9-anilinoacridine, pyronaridine (**6**) was first synthesized in China in 1970. It is a blood schizontocidal drug that is structurally related to chloroquine [33]. Pyronaridine is clearly the most active 4-aminoquinoline derivative and is generally active against chloroquine resistant parasites [34, 35]. Mechanism of action of pyronaridine is still not known. Most likely it acts through inhibition of polymerization similar to that of other 4-aminoquinoline derivatives [36, 37]. Kimberly *et al.* newly reported a series of novel 7-chloro-4-aminoquinoline (**7**) derivatives exhibiting high *in vitro* activity against four different strains of *P. falciparum* [38].

b) 8-Aminoquinolines

8-aminoquinolines were discovered during 20th century. This class of antimalarials is active against multiple life-cycle stages of the genus *Plasmodium*. Paul Ehrlich the "father of chemotherapy" in 1891 observed selective uptake and staining of tissues by dyes such as methylene blue (**8**). On this basis he hypothesized that this selectivity is may be due to presence of some specific receptors to which the dye binds and affects the parasite. This finding lead to the development of 8-aminoquinolines [39, 40]. Today it is known that methylene blue inhibits glutathione reductase thereby disturbing

the redox homeostasis of the parasite [41, 42]. Pamaquine (**9**) was synthesized by German synthetic chemist in 1925. This 8-aminoquinoline was the first drug that was capable of preventing the relapses in *P. vivax* malaria. Another 8-aminoquinoline is primaquine (**10**) which has already been used since the 1940s for the eradication of liver stages in course of *P. vivax* infections. It is the only tissue schizonticide (exoerythrocytic) drug available for radical treatment of *P.vivax* or *P. ovale* infections. In addition it inhibits the maturation of fertile gametocytes [43]. Araujo *et al.* synthesized imidazolidin-4-one derivatives (**11**) of primaquine and reported that it inhibits the development of the sporogonic cycle of *P. berghei* and they considered these derivatives as a novel type of 8-aminoquinoline antimalarial [44]. A new 8-aminoquinoline, tafenoquine (WR-238605) (**12**) is now in clinical trials and may revolutionize the prevention of malaria in travelers. It is an improved derivative of primaquine and pamaquine [45] and being a new long-acting 8-amino-quinolone it can be utilized for malarial chemoprophylaxis in geographic areas with chloroquine-resistant *P. falciparum* and *P. vivax* malaria. Tafenoquine is having the potential to kill all forms of the parasite and with its long elimination half-life, this drug may have role during malaria epidemics when mass administration is implemented [46]. The mode of action of the 8-aminoquinolines is largely unknown [47].

c) Antifolates

Besides chloroquine the antifolate combination sulfadoxine-pyrimethamine (Fancidar®, S/P) is the most common medication for treatment of malaria [48, 49]. But chloroquine resistant *P. falciparum* is found to be resistant to sulfadoxine-pyrimethamine combination. In combination with sulfadoxine-pyrimethamine or alone Tagbor *et al.* assessed the safety and efficacy of amodiaquine as an alternative regimen. They found that amodiaquine alone or in combination with sulfadoxine-pyrimethamine, although associated with minor side-effects, is effective when used to treat malaria in pregnancy [50]. Pyrimethamine (**13**) inhibits dihydrofolate reductase while the sulfonamide compound sulfadoxine (**14**) inhibits dihydropteroate synthase, an essential enzyme of the folate metabolism [51, 52].

Under a prophylactic regimen, occasional hypersensitivity reaction to the sulfonamide component may give rise to a toxic epidermal necrolysis, known as Stevens Johnson's syndrome and for this reason the approval of sulfadoxine-pyrimethamine has been retracted in several industrialized nations [53]. Another frequently used antimalarial drug is proguanil (**15**), a biguanide and its antimalarial activity was first described in 1945. It was regarded as the prodrug which is transformed into cycloguanil, a further inhibitor of dihydrofolate reductase by a cytochrome p-450 dependent reaction [54]. Proguanil showed some intrinsic activity against falciparum malaria [55].

2.2. Oxygen Containing Heterocyclic Compounds

a) Peroxides

The spread of the multi drug resistant *P. falciparum* highlighted the urgent need to develop new antimalarial drugs. Structural confirmation of artemisinin (**16**) in 1972 has provided the impetus for the investigation of the derivatives of this novel non-alkaloidal compound. Artemisinin meets the dual challenges posed by drug resistant parasite and the rapid progression of the malarial illness [56]. It is a sesquiterpene lactone that contains a 1,2,4-trioxane ring system. The antimalarial activity of artemisinin involves a cleavage of the endoperoxide linkage of artemisinin by the heme iron. Within this step, an oxygen free radical is produced, which is subsequently rearranged to give a carbon free radical. The second step, the alkylation step, involves an alkylation of specific malarial proteins by the carbon free radical, which causes a lethal damage to malarial parasite [57, 58]. It was observed that there is an apparent association between the peroxide functional group and antimalarial activity. A substantial effort has been made to develop new peroxidic antimalarials that resulted in the discovery of several new potent antimalarial compounds. Derivatives of artemisinin, artemether (**18**), artesunate (**19**), arteether (**20**), artelinic acid (**21**) are currently in use [59, 60]. In the present scenario artemisinin and its derivatives are a promising new class of drugs. Combination therapies with artemisinin derivatives with longer prophylactic time significantly lower patient's infectiousness and have the potential to reduce transmission of the parasite in uncomplicated cases of *P. falciparum* malaria [61]. It is believed that combination therapy (CT) is an effective approach in curbing the development of resistance of parasites to either compound especially if the activities of the compounds are unrelated, preferably compounds with different modes of action [62, 63]. With respect to their rapid onset of action, artemisinin derivatives are superior to any other antimalarial drugs. Moreover there has been no clinical report on artemisinin resistance. The potentially toxic dihydroartemesinin (**17**) was generated by hydrolysis of artemisinin derivatives [64, 65]. They have a short elimination half-life (2-3 hours), which means that they carry a smaller risk of developing resistance. Therefore considerable efforts have gone into the design of more stable derivatives with improved bioavailability. With particular respect to intravenous treatment of severe malaria, artelinic acid, which is significantly more stable than sodium artesunate and highly soluble in water, has been developed. Consequently, Yingzhaosu-A (**22**) which similarly to artemisinin was isolated from a traditional Chinese medicinal plant (*Artabotrys uncinatus*) and the synthetic derivative arteflene (**23**) are active against malaria. However, its moderate clinical efficacy and lack of synthetic feasibility prevented the further development of arteflene [66].

The *p*-trifluoromethyl phenoxy derivative (**24**) proved to be superior to artesunate after oral application in malaria infected mouse model [67]. Additionally robust and orally active derivatives such as (**25 a**,**b**,**c**), were obtained by nonacetal linkage of the side chain to the artemesinin core [68]. It was further rationalized that a piperazine ring in the side chain of artemisinin may lead to enrichment of the drug inside the food vacuole mediated by the weak base effect. Thus in mice infected with *P.berghei*, (**26**) was twice as active as artemether [69].

To obtain new clinically effective candidate against resistant species, trioxaquines were synthesized with an aim of combining the pharmacological advantage of the artemisinin like peroxides and the 4-amino quinolines. These compounds

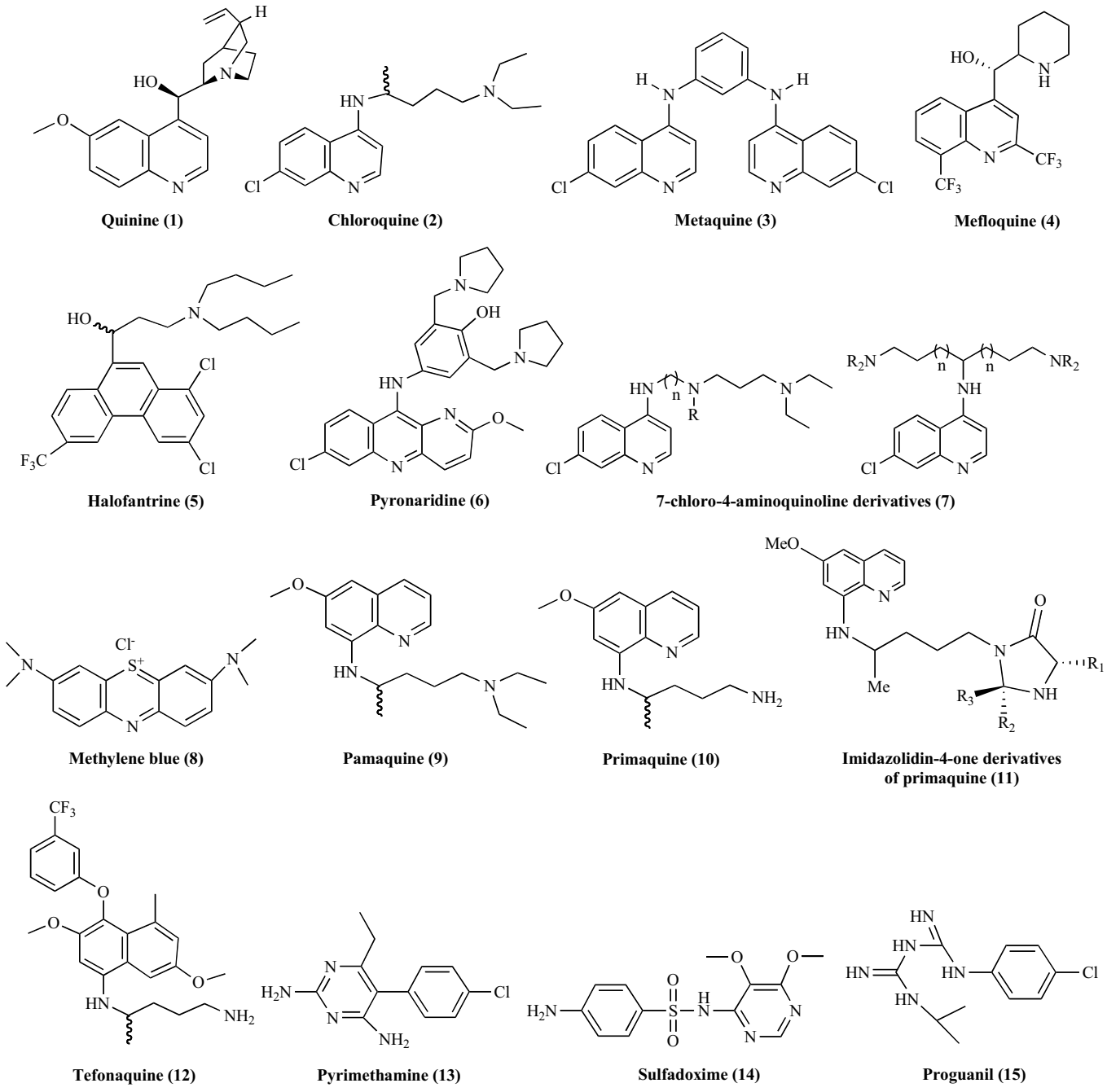


Fig. (2a). Nitrogen containing heterocyclic compounds.

are covalent conjugates of a 4-amino-quinoline entity with a trioxane motif and thus having two pharmacophores that could interact efficiently with the heme [70].

Trioxaquines combine a Fenozan-type trioxane and a 4-aminoquinoline in a single hybrid structure. Compound (**27**) obtained by reductive amination reaction of a trioxane ketone with the corresponding quinoline primary amine was found to be active when administered orally in infected mice [71]. In another such studies few trioxaquines were synthesized by Benoit-Vical and coworkers and they found that trioxaquines are highly potent against young erythrocytic stages of *P. falciparum* and exhibit efficient activity *in vitro* against chloroquine-sensitive and -resistant strains of *P. falciparum*. The most promising molecule among these trioxaquines is DU1302 (**28**). Additionally it also exhibits potent activity against gametocytes, the form transmitted by mosquitoes and thus having the potential to limit the spread of malaria [72].

Recently Cosledan *et al*. reported the synthesis and antimalarial activities of another hybrid trioxaquine molecule named PA1103/SAR116242 (**29**). This particular molecule was selected for preclinical development as an orally active drug candidate. It exhibited high activity *in vitro* on several sensitive and resistant strains of *P. falciparum* at nanomolar

concentrations (IC50 value of 10 nM with a chloroquine resistant strain, FcM29). The compound exhibited dual mode of action: heme alkylation *via* the reductive activation of the trioxane entity and the aminoquinoline inhibiting the formation of hemozoin by π-π stacking [73].

Further it has been confirmed that the alkylation capacities of trioxaquines in mammals infected with *Plasmodium* strains are similar to that of artemisinin [74]. Few chimeric molecules consisting of two pharmacophores, reported by Opsenica *et al.* consisting of tetraoxane and 7-chloro-4-aminoquinoline were referred to as "tetraoxaquines". The amide spirocyclohexylidene tetraoxaquines (**30**) was found to be more potent than the corresponding steroidal analogue (**31**) against three strains of *P. falciparum*: D6 (chloroquine-susceptible), W2 (chloroquine-resistant, susceptible to mefloquine), and TM91C235 (a multidrug-resistant strain).The amines dicyclohexylidene (**32**) is equipotent to artemisinin [75].

b) 1,2,4-trioxanes

On the basis of the pharmacophore present in the form of linked endoperoxide, Sabbani *et al.* synthesized several novel piperidine dispiro-1,2,4-trioxanes. All the compounds showed nanomolar antimalarial activity against the 3D7 strain of P. falciparum *in vitro* when compared with 1,2,4,5-tetraoxane and 1,2,4-trioxolane as standard. The amine hydrochloride intermediate (**33**) exhibited highest potency with an IC_{50} of 120 nM and the phenylsulfonyl derivative (**34**) showed moderate activity of 710 nM. But the regioisomer of (**34**) phenylsulfonyl 1,2,4-trioxane (**35**) was inactive at a concentration as high as 1000 nM [76]. Recent preclinical evaluation of a water-soluble synthetic trioxane, 3-carboxyphenyltrioxane (**36**) in rodents exhibited good therapeutic index (efficacy/toxicity) at high dose as that of artelinic acid [77].

c) Steroid-based 1,2,4-trioxanes

Some of the steroid-based 1,2,4-trioxanes synthesized and evaluated by Singh *et al.* showed very significant activity against multi-drug resistant *P. yoelii* in Swiss mice by oral route. The size and nature of the steroidal side chain governs the activity. Pregnane-based trioxanes (**37**) showed better activity profile than trioxanes (**38**) and (**39**) which are derived from cholesterol and tigogenine, respectively. Among pregnane-based trioxanes, (**37b**) and (**37f**) were the most active compounds of the series [78]. Very recently the same group evaluated a series of new amino and ester functionalized 1,2,4-trioxepanes on the same mice model taking β-arteether as the standard. Amino functionalized trioxepanes (**40**) emerged as the most active antimalarial compound of the series [79].

d) 1,2,4 Trioxolanes/Secondary Ozonides

Due to the lack of ideal "drug likeness" of artemisinins and their semisynthetic derivatives Vennerstrom and co-workers developed a promising drug candidate OZ277 or Rbx-11160. This particular candidate exhibits structural simplicity along with superior pharmaco-kinetic and -dynamic properties comparable to their natural counterparts. OZ277 (**47**) emerged as a potentially low toxic and active drug among the other synthesized dispiro-1,2,4-trioxolanes (**41-46**) . The compounds (**41&42**) where the peroxide bond is sterically inaccessible to iron (II) species for iron (II)-mediated fragmentation and generation of free radicals were completely inactive. But in the case of rest of the compounds formation of free radical resulting from attack of the Fe(II) species on the less-hindered peroxide bond oxygen atom was observed as exemplified by the β-scission of the spiroadamantane ring and the free radical was trapped by the stable nitroxide free radical 2,2,6,6-tetramethyl-1-piperidine-1-oxyl (TEMPO) (**44**) [80].

The spiroadamantane ring protects the endoperoxide bridge and the side chain ($CONHCH_2C$ $(CH_3)_2NH_2$ of OZ277 is responsible for the compound's improved water solubility and pharmaceutical properties [81]. OZ277 acts by alkylation of specific parasite proteins, PfATP6, a sarcoplasmic reticulum calcium ATPase, the proposed target for semisynthetic peroxidic artemisinin derivatives [82]. Recently the drug candidate has been reported to enter Phase IIb dose range studies in India, Thailand and Africa after the successful completion of Phase IIa study [83]. Further development of a fixed dose combination of piperaquine with Rbx11160 by Ranbaxy and MMV (Medicines for malaria venture) team is under progress. Parallel investigations performed by Snyder and coworkers reported a synergistic interaction of Rbx11160 with piperaquine with no signs of toxicity [84]. The promising pharmacophore i.e. achiral dispiro-1,2,4-trioxolane flanked by a spiroadamantane and spirocyclohexane was identified as a lead compound. Later Dong and coworkers synthesized a number of trioxolanes and the compound (**48**) showed the least metabolic liabilities (low plasma clearance) with good bioavailability. It was found that trioxolanes were substantially less effective in a standard oral suspension formulation compared to a solubilizing formulation and were more active subcutaneously than orally [85]. They further confirmed that the more lipophilic trioxolanes had better oral activity than their more polar counterparts and trioxolanes with a wide range of neutral and basic, but not acidic, functional groups had good antimalarial profiles [86].

Several weak base dispiro-1,2,4-trioxolanes were synthesized and compared with the antimalarial profiles of compound OZ209 (**46**) to identify a potent weak base analog of (**46**) with high oral activity, good biopharmaceutical properties, and low toxicity. After assessment of *in vitro* and *in vivo* antimalarial activities it was established that for synthetic 1,2,4 trioxolanes and its analogs, spiroadamantane ring system essentially attributed to the antimalarial properties. Among all the prepared bases none was found to have superior activity profile to that of primary amine (**46**) but compounds (**49**) & (**50**) were less toxic. In addition good antimalarial profiles shown by weak base azole (**38**) suggested that trioxolanes do not require an aliphatic amino functional group for high antimalarial activity [87].

e) Tetraoxane- Based Antimalarial Compounds

Endoperoxide based antimalarials appeared in various literatures [88, 89]. They attracted a lot of attention from medicinal chemist world over because of their easy synthesis and low cost of raw materials. First series of compounds

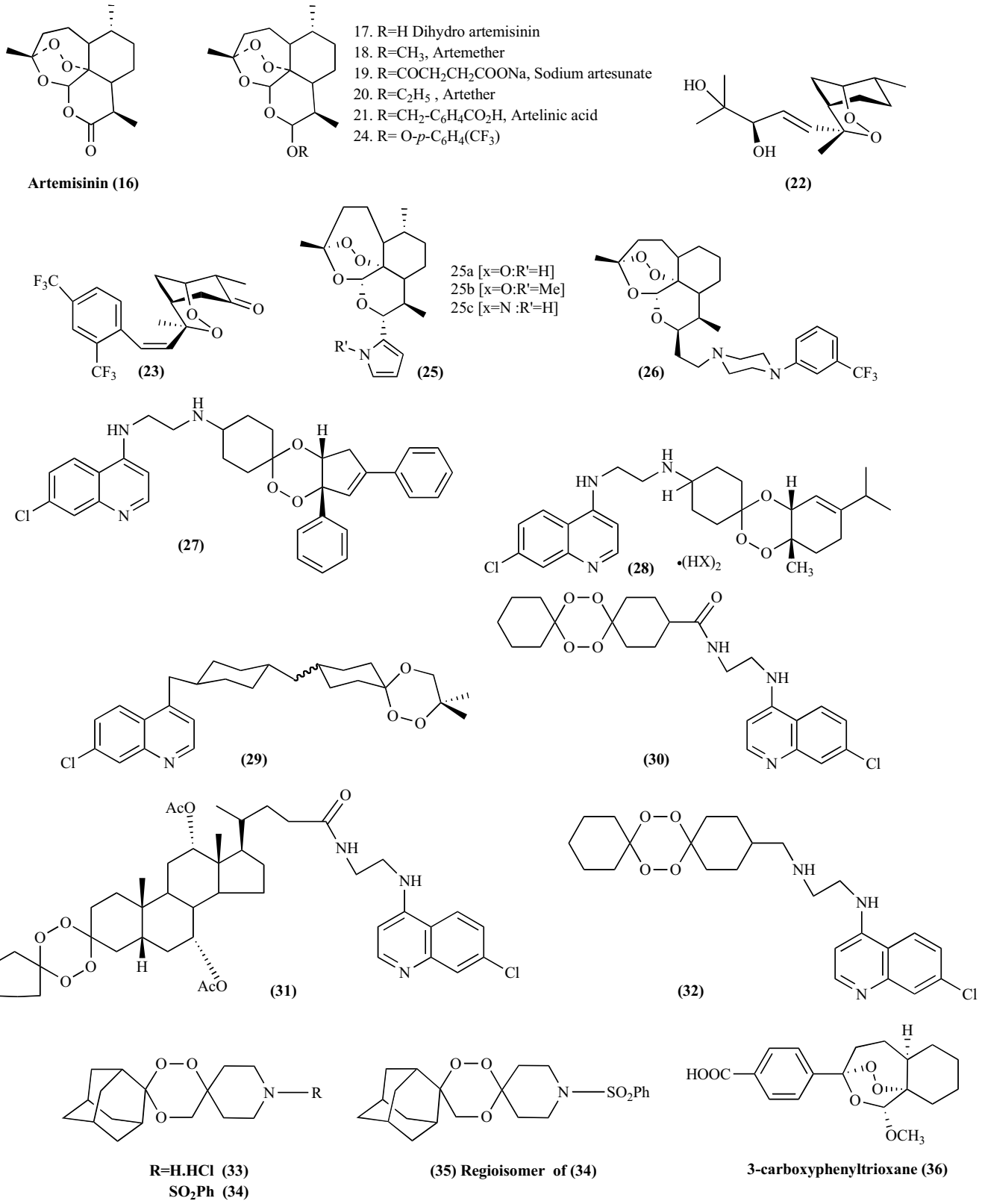


Fig. (2b). Oxygen containing heterocyclic compounds.

came to existence in 1992 when Vennerstorm *et al.* synthesized following three compounds and confined their *in vivo* antimalarial activity against *P.falciparum* [90, 91]. It was observed that the compounds (**51**) and (**52**) are 2 and 26 fold less potent respectively than artemisinin, but compound (**53**) was found to be as potent as artemisinin. Thus, they con-

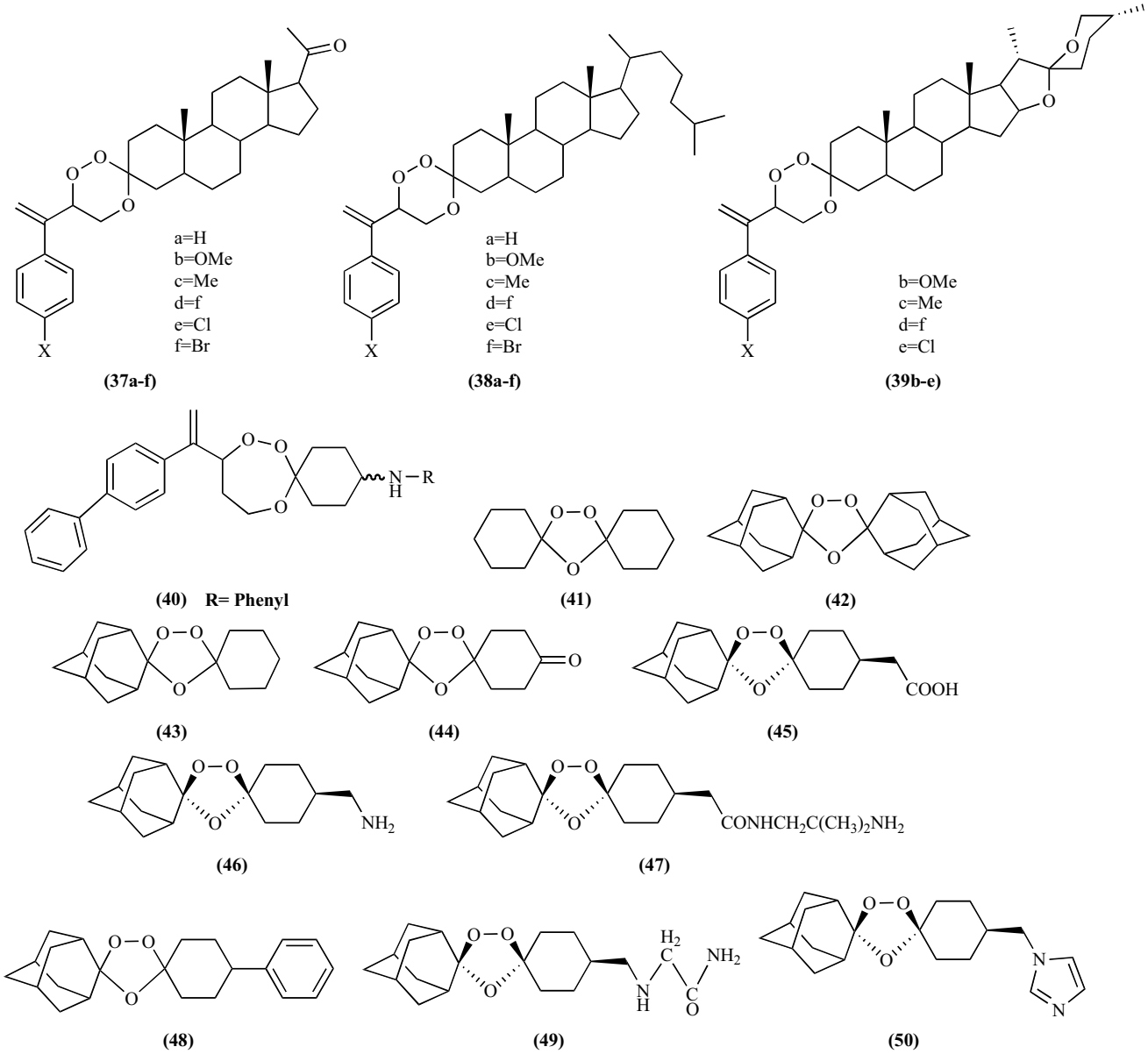


Fig. (2c). Oxygen containing heterocyclic compounds.

firmed the curative power of 1,2,4,5 tetraoxanes. In the same year, Vennerstorm *et al.* filed a patent under the title of "Novel antimalarial peroxide and process for their production and use". Then onwards compound (**53**) or WR148999 become the prototype for the synthesis of new compounds of tetraoxane series. Based upon this prototype, various modifications were done to the dispiro–1, 2, 4, 5-tetraoxanes by substituting unsaturated and other polar functionalities at 1 and 10 positions to improve water solubility of these very lipophilic tetraoxanes (Table **1**) [92-94].

MC Cullough *et al.* in 2000 showed that the completely unsubstituted dispiro 1, 2, 4, 5-tetraoxane was as potent as the prototype (**52**). Thus, the compound (**53**) became an important pharmacophore for the synthesis of a new class of tetraoxane antimalarials [95-97]. In the year 1996, steroidal geminal dihydroperoxides were synthesized and tested for their biological activity against malaria [98]. Out of the synthesized compounds only bis (3-dioxy -5α-cholestane) was found to have activity in the range of most potent recently synthesized arteether glucoronides. With all this work, it took no time for 1,2,4,5,-tetraoxane being recognized as an increasingly interesting pharmacophore. It was found to be very similar to 1, 2, 3 trioxanes such as naturally occurring artemisinin and its semi synthetic derivatives and related compounds. Therefore, several efforts have been directed toward synthesis of these kinds of endoperoxide antimalarial compounds and correlation of antimalarial activity seen *in vivo* and *in vitro*. Solaja *et al.* synthesized cholic acid derivatives as carriers of 1, 2, 4, 5 tetraoxanes [99]. The compounds synthesized showed that the ester moiety as well as the corresponding acids are inactive against D6 and W2 strains of the parasite. The amide introduced for the first time as an auxiliary functional group in a tetraoxane mole-

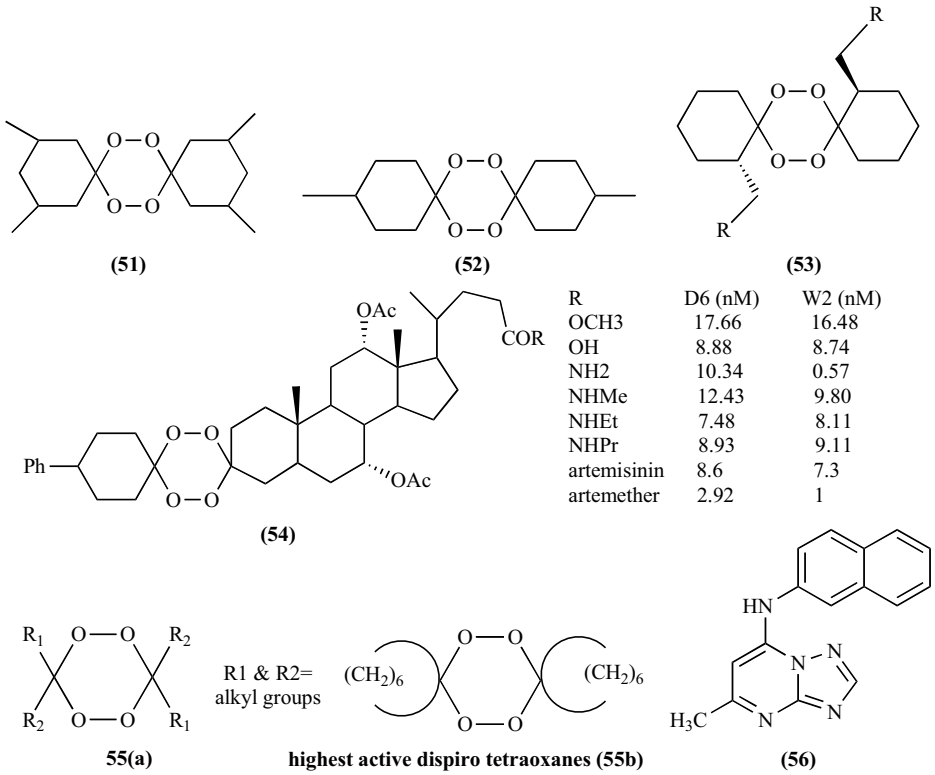


Fig. (2d). Oxygen containing heterocyclic compounds.

cule increases the antimalarial activity with no RBC membrane lysis, suggesting that antimalarial activity is the consequence of interaction specific to infected RBC, and is not the result of uncontrolled RBC membrane lysis. Mixed tetraoxanes of the phenyl-substituted "cyclohexyl-spiro tetraoxa cyclohexyl-spirocholate" series (**54**) has been prepared by Solaja *et al.* and evaluated as possible antimalarial, anti tubercular, anti proliferative activity [100, 101].

In 2006 Zmitek *et al.* efficiently synthesized novel 3,3,6,6-tetraalkyl-1,2,4,5-tetraoxanes (**55a**) directly from cyclic, as well as the less reactive acyclic ketones using fluorinated alcohols and evaluated their *in vitro* antimalarial activities against chloroquine-resistant FCB1 strain of *P. falciparum*. The simple structure of the compounds enables them to study the influence of ring size and alkyl chain length keeping the properties of pharmacophoric peroxide unit intact. Among the synthesized 3,3,6,6-tetra-alkyl-1,2,4, 5-tetraoxanes, the dispiro tetraoxane (**55b**) showed the highest antimalarial activity. The study reveals that the extension of the alkyl chain in tetraoxanes does not influence the electronic properties of the peroxide bond as alkyl chains have similar electron-donating abilities. They suggest that the more lipophilic a compound the better is the antimalarial activity [102].

Target-based drug discovery has been put forth as a promising mechanism for the discovery of new drugs. Dihydroorotate dehydrogenase (DHODH) inhibitors by high-throughput screening (HTS) that have potent antimalarial activity provide a thriving example. Triazolopyrimidines (**56**) exhibit good association between *P. falciparum* dihydroorotate dehydrogenase (*Pf*DHODH) inhibitory activity and antimalarial potency in the infected erythrocyte model [103].

2.3. Miscellaneous

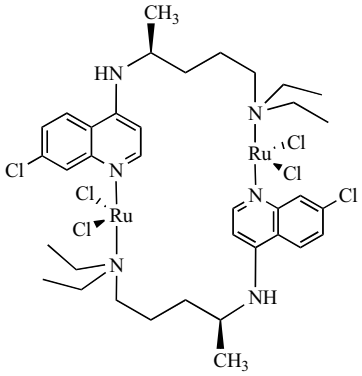
a) Metal Complexes

In the cases of tropical diseases viz. malaria, trypanosomiasis and leishmaniasis, metal ion complexes with various established drugs were studied for enhancement of pharmacological properties. Metal-drug synergism as observed with antimalarials was obtained by utilizing the three basic N-donor atoms of chloroquine base. Metal complex of ruthenium-chloroquine (**57**) served as an appropriate example in which the activity of the parental drug is enhanced by 4.5 times without any resistant phenomenon [104]. Chloroquine has also been coordinated with transition metal iridium and three new iridium-chloroquine complexes were tested *in vitro* and all of them showed antimalarial activity against *P. berghei*. The complex generated by reacting iridium chloride salts ($IrCl_3.3H_2.O$) (**58**) with chloroquine emerged as the most active compound with an IC_{50} value 59.0 nM as compared to chloroquine diphosphate [105].

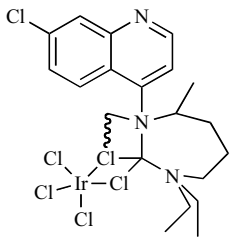
Recently a number of metal complexes of antiprotozoal drugs reported by Ajibade showed promising results. As for example the incorporation of copper into trimethoprim ($[Cu(TMP)_2(CH_3COO)_4]$) enhanced antiplasmodial activity against the resistant strain of *P. falciparum* with an IC_{50} value of 3.7231 μM [106]. A better copper (II) ion complex with an improved IC_{50} value (2.65μM) has been obtained by complexing with pyrimethamine *via* bonding through the N (1) atoms of the drug (**59**) [107].

Table 1. Comparative Activity of WR148999 Derivatives Compared with Artemisinins Standard [93]

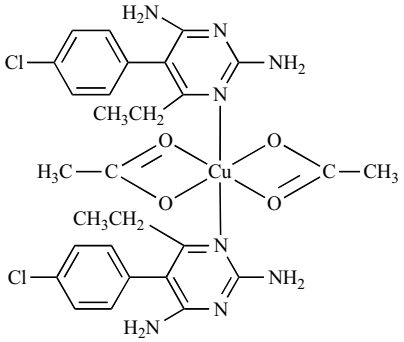
S.No	Comp.	R (refer. Str. no. 42)	IC50 (nM)		S.No.	Comp	R	IC50 (nM)	
			NF 54	K1				NF 54	K1
1.	53A	$CH=CH_2$	23	19	7.	53G	COOH	>200	>200
2.	53B	C≡CH	13	13	8.	53H	OH	>200	>200
3.	53C	C_6H_5	>200	>200	9.	53I	$OCOC_6H_5$	>200	>200
4.	53D	$CH_2COOC_2H_5$	16	14	10.	53J	OCH_3	16	15
5.	53E	$COOC_2H_5$	6.5	6.2	11.	53K	$OCH2C_6H_5$	26	18
6.	53F	CH_2COOH	>200	>200	12.	53	H	39	28
					13.	Artemisinin	-------	12	10



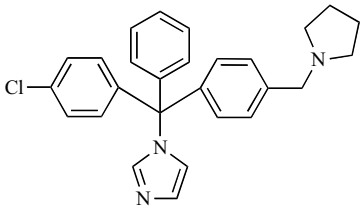
Metal complex of ruthenium-chloroquine (**57**)



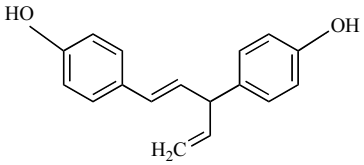
Proposed structure of iridium chloroquine complex (**58**)



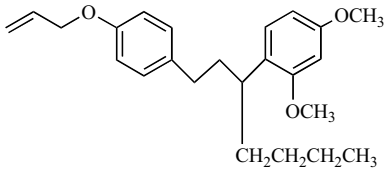
Copper (II) ion complex withpyrimethamine (**59**)



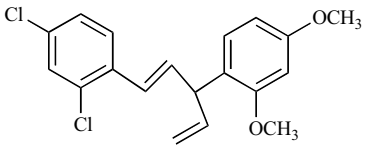
(**60**)



Norneolignan hinokiresinol (**61**)



(**62**)



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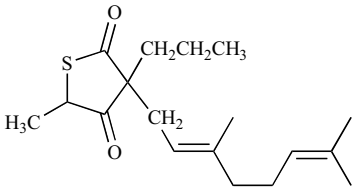

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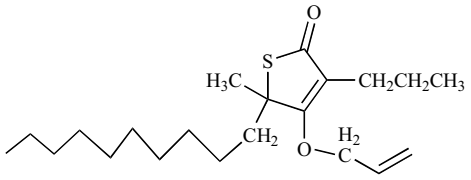

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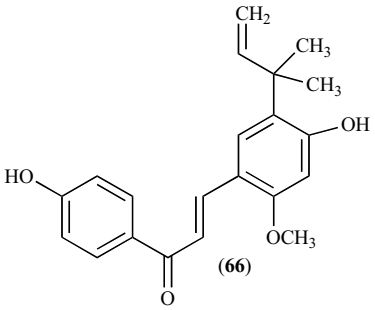

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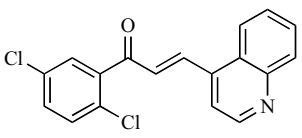
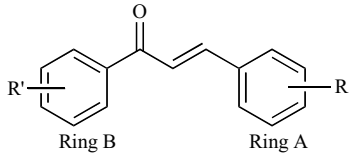


(**67**)



(**68**)

(Fig. 3). Contd….)

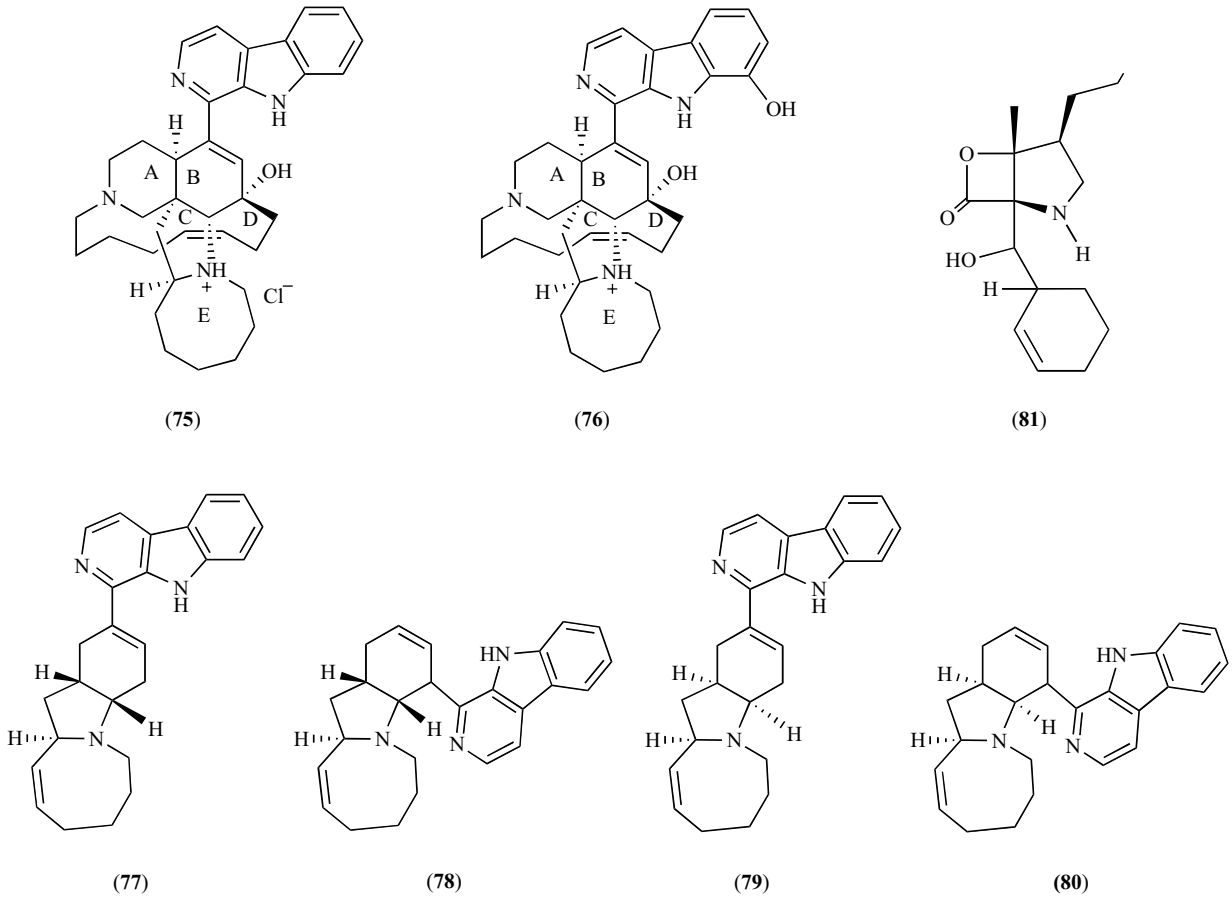


Fig. (3). Miscellaneous antimalarials.

b) Other Heterocycles

In continuation to the efforts carried out for the development of an efficient antimalarial Gemma *et al.* developed a novel polycyclic pharmacophore based on clotrimazole scaffold (Fig. (**4**)).

All the compounds were evaluated for antimalarial activities against a series of *P. falciparum* strains and they found that compounds selectively interact with free heme of the parasite. Maximum efficacy was shown by compound (**60**), elicit a potent *in vitro* antimalarial activity with low cytotoxicity [108, 109].

c) Natural Antimalarials

i) Norneolignan Hinokiresinol

A natural product isolated from a number of sources, Viz. *Chamaecyparis obtusa*, *Agathis australis*, *Libocedrus yateensis*, *Athrotaxis selaginoides*, *Cryptomeria japonica* and *Araucaria angustifolia* provided a novel template for the development of drugs against malaria. Skytte *et al.* developed an efficient method for the synthesis of hinokiresinol analogues *via* Carroll rearrangement based on the lead skeleton (**61**). Depending upon the side chain and ring substitution pattern they report two most active compounds, (**62**) and

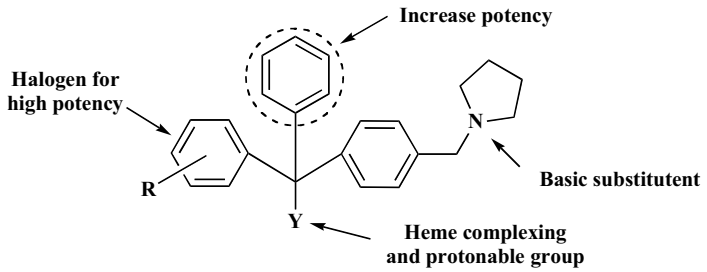


Fig. (4). Optimized Pharmacophore designed by Sandra Gemma *et al.*

Table 2. Various Substitution Patterns on the Two Aromatic Ring of Chalcone

S.No	Comp	R' (57)	R (57)	IC_{50} (μM)
1.	69	2',3',4' trimethoxy	4-trifluorophenyl	3.0
2.	70	2',3',4' trimethoxy	3-quinolinyl	2.0
3.	71	2',4' dimethoxy	2,4 dimethoxy	2.1
4.	72	2',4' dimethoxy	4-ethyl	2.4
5.	73	2',4' dimethoxy	3-quinolinyl	2.2
6.	74	4' methoxy	3-quinolinyl	4.8

(**63**) with no apparent toxicity [110, 111]. Compounds related to natural antibiotic thiolactomycin were synthesized by Simon M. Jones *et al.* Among the 4-ketothiolactones analogues, (**64**) appeared to be the most active compound as compared to thiolactomycin. But alkyloxy substitution at C4 of the thiolactone ring showed 143-fold increase in activity and the most active compound reported is (**65**) among the alkyloxy series [112].

ii) Microbial Depsipeptides

A recent review extensively highlighted a few depsipeptides primarily obtained from fungi, actinomycetes, cyanobacteria, higher plants and marine organisms. Some of the potential depsipeptides which are in various stage of clinical development are Jasplakinolide, Hirsutellide A, Beauvericin, Allobeauvericin C, Enniatin B_4 & Enniatin I having Plasmodium IC50 (μM) values of 0.32, 4.2, 1.7, 1.9, 0.31 & 0.36 respectively [113].

iii) Chalcones

The first reported chalcone (1,3-diphenyl-2-propen-l-one) to have antimalarial activity was Licochalcone A (**66**), isolated from Chinese licorice roots (Gan Cao). It was found to exhibit potent antimalarial activity that can inhibit the *in vitro* growth of the human malaria parasite [114]. In 1995 Li *et al.* screened a series of chalcones and their derivatives and they are active against both chloroquine-sensitive and chloroquine-resistant strains of *P. falciparum*. Chalcone derivative, l-(2,5-dichlorophenyl)-3-(4-quino-linyl)-2-propen-l-one (**67**) emerged as the most active compound with an IC50 value of 200 nM [115]. Varying the substitution pattern on the two aromatic rings of chalcone (**68**), various alkoxylated and hydroxylated analogues of chalcones has been generated by Liu *et al*. and they found that the ring B is important for activity (cf Table **2**). Among the reported compound, the methoxylation rather than hydroxylation of ring B at various position generated compounds with good *in vitro* activities. The promising molecules (IC_{50} < 5 μM) are trimethoxy (**69**) and (**70**), dimethoxy (**71**, **72**, **73**), and methoxy (**74**) analogues (Table **2**) [116].

In 2005 phenyl urea chalcone derivatives were synthesized and tested against chloroquine resistant strain of *P. falciparum*. From various studies it was found that chalcones exert their antimalarial activity *via* multiple mechanisms [117].

iv) Antimalarials from Marine Origin

Marine derived natural products still remain unexploited sources in the field of antimalarial drug discovery, until recently a considerable research oriented toward bioassay-guided isolation and screening of antimalarial compounds from marine organisms has been initiated. Among the potential compounds reported so far to possess antimalarial activity, majority of them are isolated from sponges. Marine derived β-carboline alkaloids manzamine-A (**75**) and its hydroxyl derivative (-)-8-hydroxymanzamine-A (**76**) isolated from various species of sponges also have significant inhibitory activity against malaria parasite both *in vitro* and *in vivo* in addition to its anticancer activity. Studies suggested that both the compounds are active against the asexual erythrocytic stages of *P. berghei* infected mice and prolong the survival period as compared to chloroquine and artemisinin treated mice. The compound is now considered as promising lead for extended preclinical assessment [118, 119].

The importance of ring A and D of manzamine-A together with the stereochemistry and cis-exo orientation of the azocine (ring E) relative to the BC ring system is considered vital for antimalarial activity and potency. The fact is exemplified by the activities of four simplified analogues (**77-80**) whereby it has been reported by Winkler *et al.* that none of the analogues were as active as manzamine A (IC_{50} 13.5 ng/mL), the parent pharmacophore. Among the synthesized analogues, compound (1) which incorporates the above structural and stereochemical requirements retains certain amount of activity (IC_{50} 310 ng/mL) [120].

Another promising inhibitor at the erythrocytic stages of the malaria parasite is salinosporamide-A (**81**). Like manzamine-A, the compound isolated from the marine actinomycete *Salinispora tropica*, showed significant cytotoxic activity against human tumor cell lines. It is a highly potent inhibitor of the human malaria parasite *in vitro* (*P. falciparum*) and *in vivo* (*P. yoelii*) at low dose. Upon phenotypic analysis of the three main intra-erythrocytic stages of the parasite-the ring, trophozoite and/or schizont stages cycle, salinosporamide A is quick and efficient in arresting cell cycle progression at the schizont stage. The molecular target is the 20S proteasome of the plasmodium parasite as determined by homology modeling and virtual ligand interaction [121].

Polycyclic quinones, xestoquinone (**82**) and halenaquinone (**83**) have been isolated from a new species of genus Xestospongia (order Haplosclerida, family Petrosiidae).On preliminary *in vitro* screening, xestoquinone was reported to be a potent (IC50 of 3 μM) inhibitor of Pfnek-1, a protein kinase of *P. falciparum*. However it exhibited a weak *in vivo* activity at 5 mg/kg in *P. berghei* NK65 infected mice and was toxic at higher doses [122]. Compounds related to xestoquinone with unique substitution on a tetrahydrofuran junction were isolated from a New Caledonian deep water sponge. The new meroterpenes, alisiaquinones (**84-86**) and alisiaquinol (**87**) displayed *in vitro* micromolar activity on two enzymatic targets of importance for the control of malaria, the plasmodial kinase Pfnek-1 and a protein farnesyl transferase (PFTase). Among the meroterpenes, Alisiaquinone C exhibited a submicromolar *in vivo* activity on different strains of *P. falciparum* [123].

Plakortin (**88**), the major cycloperoxide extracted from the marine sponge *Plakortis simplex* [124] served as an outstanding novel template for the design of semisynthetic endoperoxide antimalarials. Based on the activities of a series of compounds using plakortin as the pharmacophore, structural features (Fig. **5**) necessary for activity has been suggested. The absolute inefficacy of diol compound (**89**) revealed the importance of endoperoxide skeleton. Substitution pattern on ester functionality has no impact on the activity but the changes on the 'western' alkyl chain determine the activity and has crucial role in governing the potency. Esterification of carboxylic function (**90**) is critical for effective parasite penetration as free carboxy function result in decrease in activity (**91**) [125].

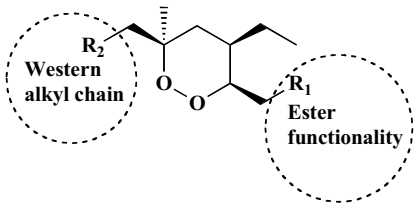


Fig. (5). Structural features of plakortin analogues.

v) Double Drug Approach: Novel Plasmepsins I and II Inhibitors

New antimalarial compounds that acts through distinct mechanisms during both the liver and the blood stages of the parasite life cycle were being developed by Agli *et al*. Compounds were designed on the basis of the "double-drug" approach: primaquine, which has been linked to statine-based inhibitors of plasmepsins (PLMs): PLM I and PLM II, the plasmodial aspartic proteases involved in degradation of hemoglobin. The most promising compounds (**92**, **93**, **94**) showing nanomolar potency were not cytotoxic against human fibroblasts and were highly selective for PLMs *vs* human cathepsin-D [126]. A review elaborately addressing the parasite's hemoglobin metabolism and numerous plasmepsin aspartic proteases inhibitors has been published elsewhere [127].

3. DEVELOPMENT OF DRUG RESISTANCE

Antimalarial drug resistance has been defined as the "ability of a parasite strain to survive and/or multiply despite the administration and absorption of a drug given in doses equal to or higher than those usually recommended but within tolerance of the subject" [128]. This definition was later modified to specify that the drug in question must "gain access to the parasite or the infected red blood cell for the duration of the time necessary for its normal action" [129]. Although much work has already been done and the world wide research for the new drug is going on, but because of the rapid development of drug resistance, all efforts have gone in vain. Drug resistance is now considered as one of the major factors contributing to the resurgence of malaria and malaria-induced morbidity and mortality [130].

Resistance to the most affordable drugs such as chloroquine and Fansidar®, a combo-drug of pyrimethamine and sulfadoxine is worsening the case especially in developing countries. Antimalarial drug resistance greatly impairs clinical interventions with medicines and continues to undermine malaria control efforts [131, 132]. The rapid spread of antimalarial drug resistance over the past few decades has required more intensive monitoring of drug resistance to ensure proper management of clinical cases and early detection of changing patterns of resistance so that national malaria treatment policies can be revised where necessary. Recent efforts to scale-up malaria control in endemic countries throughout the world including increased support for commodities and health systems, as well as the proposed price subsidy on ACTs is resulting in greater access to and a vastly increased use of antimalarial medicines, in particular ACTs. This is leading to a much higher degree of drug pressure on the parasite which will almost certainly increase the likelihood of selecting for resistant parasite genotypes. Recent genetic and genomic advances have paved the way for discoveries into the origins and spread of antimalarial drug resistance and the underlying molecular mechanisms [133].

Mechanisms of such resistance involves the mutations in the parasite dihydrofolate reductase and dihydropteroate synthase genes conferring resistance to pyrimethamine and sulfadoxine, respectively, and by the recent discovery of mutations in the gene coding for a putative transporter, PfCRT, conferring resistance to chloroquine [134].

In the present scenario, there are no effective alternatives of artemisinins for the treatment of *P. falciparum* malaria either on the market or towards the end of the development pipeline. The contribution of under-dosing to antimalarial treatment failure has been underappreciated. Most recommended dosage regimens are based on studies in non-pregnant adult patients. Young children and pregnant women, who bear the heaviest brunt of malaria burden, have the highest treatment failure rates. This has been attributed previously to lower immunity, although blood concentrations of many antimalarial drugs are significantly lower in pregnant women and young children than in non-pregnant adults. Nevertheless, there have been no studies of higher dosages regimens. Sub-therapeutic concentrations will certainly contribute to poorer responses to treatment and will fuel the emergence and spread of antimalarial drug resistance [135]. A review of a publicly-accessible central database that would contain information on the levels of resistance to currently available antimalarials like chloroquine and sulfadoxine-pyrimethamine, and on the many combinations and success-

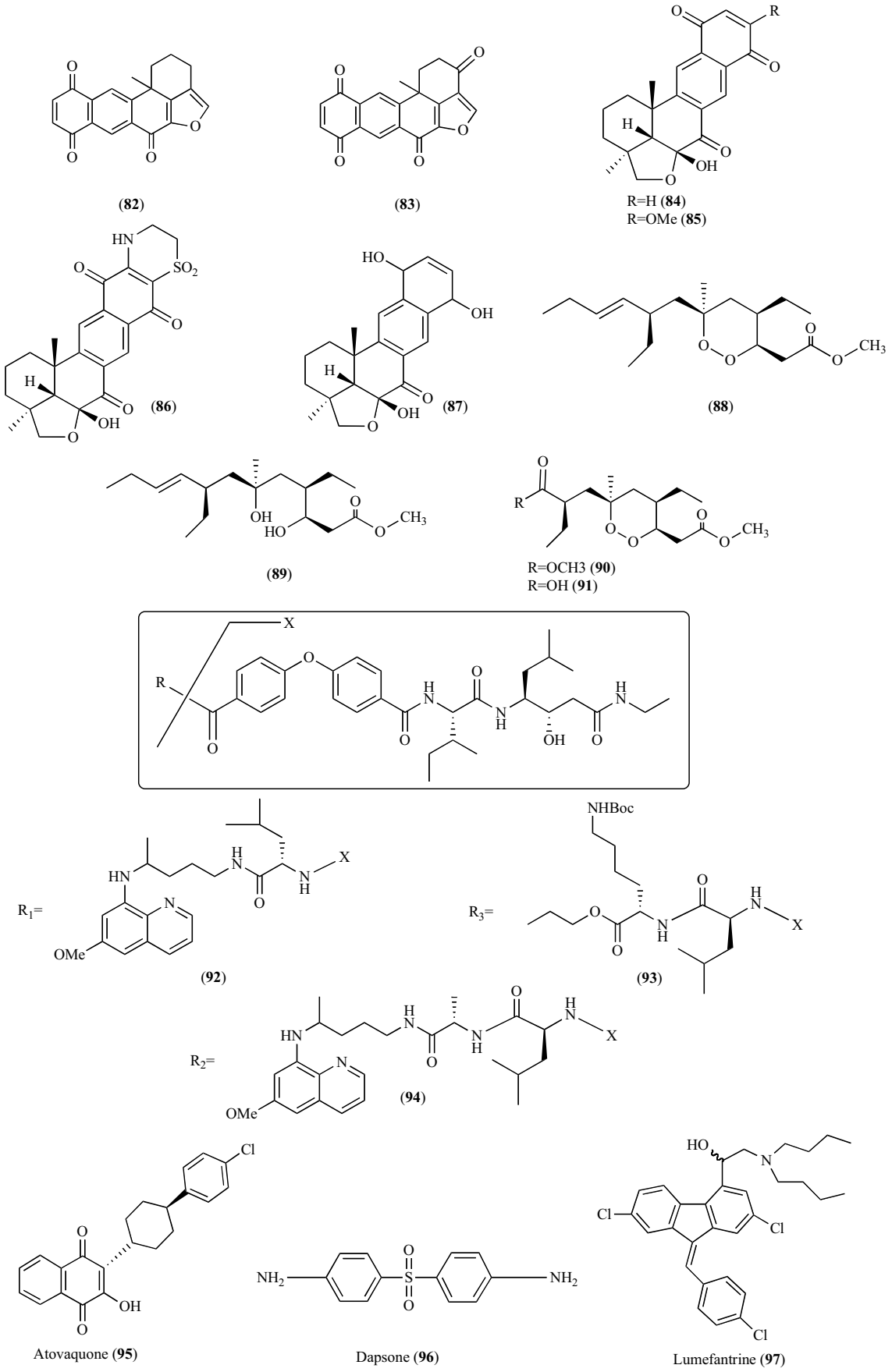


Fig. (6). Some more antimalarials.

Table 3. Past to Present Scenario of Antimalarial Drug Development

S. No	Year	Drug	Class	Source	Remarks	Targets
1	1820	Quinine	Alkaloid	C	- Hemolytic anemia in patients with G6PD deficiency	- Inhibition of hemozoin biocrystallization, thus facilitating an aggregation of cytotoxic heme
2	1926	Pamaquine	8-aminoquinoline	A	- Hemolytic anemia in patients with G6PD deficiency	- Generating reactive oxygen species or interfering with the electron transport in the parasite.
3	1931	Quinacrine	Acridine	A	- Also used in systemic lupus erythematosus	Act against the protozoan's cell membrane.
4	1934	Chloroquine	4-aminoquinoline	A	- Adversely effect immune system - 1950 resistance appears	- High alkaline pH in food vacuoles of the parasite
5	1935	Sulfadoxine	Sulphonamide	A	- Serious (possibly fatal) allergic reactions	- Inhibits dihydropteroate synthase (DHPS, EC 2.5.1.15 - Key enzymes in the biosynthesis of folate
6	1945	Proguanil	Biguanide	A	- Mouth ulcers, skin rashes, reversible hair loss, Severe kidney impairment	- Inhibiting the enzyme, dihydrofolate reductase
7	1950	Primaquine	8-aminoquinoline	A	- Effective against the gametocytes - Hemolytic anemia in patients with G6PD deficiency	- By generating reactive oxygen species or by interfering with the electron transport in the parasite
8	1951	Cycloguanil	Biguanide	A	- Active metabolite of proguanil	- Acts specifically on *P. falciparum* DHFR (EC 1.5.1.3)
9	1970	Amodiaquine	4-aminoquinoline	A	- Agranulocytes and hepatic disorders	- Inhibit heme polymerase activity - Accumulation of free heme
10	1970	Pyrimethamine	Diamino pyrimidine	A	- Adversely effect immune system - Bone marrow depressants - Megaloblastic anemia if used with other folate antagonists - Hepatic function impairment	- Folic acid antagonist
11	1970	Pyronaridine	9-anilinoacridine	A	- Effective against chloroquine-resistant parasites	- Blood schizontocidal
12	1970	Lumefantrine (benflumetol)	Fluorenemethanols	A	- Artemisinin-based combination therapy	Forming toxic complexes with ferritoporphyrin
13	1971	Mefloquine	Arylamino alcohol	A	- Negative inotropic effects - Prevention and treatment of malaria	- Blood schizonticide. Its exact mechanism of action is not known.

(Table 3. Contd….)

S. No	Year	Drug	Class	Source	Remarks	Targets
14	1971	Artemisinin Artesunate Artemether Arteether Dihydroartemisinin Artelinic acid Artenimol	sesquiterpene lactones	C B	- Water-soluble (most active and the least toxic) - lipid-soluble (longest life but also the most toxic) -Oil soluble	- Inhibiting a *P falciparum*-encoded sarcoplas- mic-endoplasmic reticulum calcium ATPase
15	1980s	Halofantrine	9-phenanthrine methanol	A	- Cardiac arrhythmia - Short half life	- Forming toxic complexes with ferritoporphyrin IX that damage the membrane of the parasite
16	1980s	Atovaquone	1,4 naphthoquinone	A	- Only available as a fixed preparation with proguanil (Malarone)	- Site of action cytochrome bc1 complex (Complex III - Inhibition of mitochondrial electron transport
17	2007	Tafenoquine (WR-238605) (Under development)	8-aminoquinoline	A	- long half-life - Use for Malaria Prophylaxis - Hemolysis in people with G-6-PD deficiency	- Sporontocidal and gametocytocidal activity

A=Synthetic, B=Semisynthetic, C=Natural.

ful implementation and subsequent utilization of such database would tremendously assist in the malaria eradication programme [136].

Numerous factors contributing to the advent, spread, and intensification of drug resistance exist, although their relative contribution to resistance is unknown. Factors that have been associated with antimalarial drug resistance include such disparate issues as human behavior (dealt with in details elsewhere), vector and parasite biology, pharmacokinetics, and economics [137, 138].

4. ALTERNATIVE APPROACHES

4.1. Combination Therapy

Multidrug resistance has been reported from most parts of the world and as a result monotherapy or some of the available combination chemotherapies for malaria are either ineffective or less effective. Antimalarial combination chemotherapy is widely advocated for delaying the development of resistance to the remaining armory of effective drugs. The rationale for using drugs in combination is well established in the treatment of tuberculosis, HIV, and cancer.

The concept of combination therapy is based on the synergistic or additive potential of two or more drugs, with independent modes of action and different biochemical targets in the parasite. Major challenges exist in the deployment and use of antimalarial drug combination therapies are the appropriate choice of drug combinations as irrational combination often leads to ineffectiveness [139].

4.1.1. Non-Artemisinin Based Combinations Table (4)

a. Atovaquone-Proguanil

Malarone™ is a fixed-dose combination of the antimalarials atovaquone and proguanil hydrochloride [140, 141]. Atovaquone, (**95**) an analogue of coenzyme Q (Ubiquinone), acts on the plasmodium cytochrome B, where it interrupts electron transport and causes loss of mitochondrial membrane potential [142, 143]. Proguanil exerts its effect by means of the metabolite cycloguanil, a dihydrofolate reductase inhibitor. It also accelerates the loss of mitochondrial membrane potential. Atovaquone/proguanil acts synergistically against the malarial parasite. This combination also brings about enzymatic inhibition of pyrimidine synthesis. When atovaquone is used alone resistance emerges very rapidly owing to mutation of the cytochrome-B gene localized in the apicoplast genome. This problem was solved by combining with proguanil [144, 145].

b. Chlorproguanil-Dapsone

Chlorproguanil-dapsone (Lap-dap®) is a recently released antimalarial drug in which two long-established compounds are formulated in a fixed combination [146]. Similar to

proguanil, chlorproguanil, transformed into the dihydrofolate reductase inhibitor Dapsone (**96**), a sulphone, has been the mainstay of leprosy treatment for over half a century. Like other sulfa drugs, dapsone inhibits dihydropteroate synthase in the malaria parasite. The two compounds thus provide sequential blockade of folate synthesis and are highly synergistic. This widely used antifol-sulfa combination provides potential advantage as the combination inherits short plasma half life of both the drugs [147]. Thus the parasites are exposed to sub therapeutic concentrations for short time only, thereby deterring the emergence of resistance [148, 149].

Artemisinin-based combination treatments (ACTs) are now generally accepted as the best treatments for uncomplicated falciparum malaria [150]. Artemisinin based combinations are known to improve cure rates, reduce the development of resistance and they might decrease transmission of drug-resistant parasites. Artemisinins are a particularly effective partner drug because they are more active than any other antimalarial in reducing the number of parasites (Fig. (**7**)) [151].

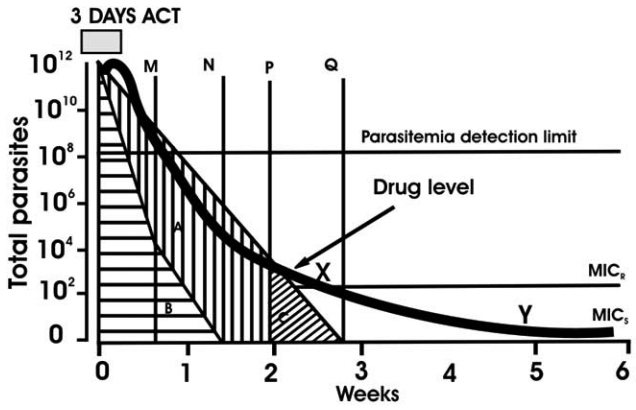


Fig. (7). Effect of combination of artemisinin with mefloquine.

Table 4. Non Artemisinin Based Combinations

S. No.	Type	Advantages	Disadvantages	Dose	Status
1	Sulfadoxine-pyrimethamine (SP)	Cheap	Drug resistance serious adverse effects	Sulfadoxine 25mg/kg Pyrimethamine 1.25mg/kg (single dose)	Not approved Considered as single drug
2	SP + Chloroquine	Cheap; varied modes of action on different biochemical targets in the parasite	Drug resistance serious adverse effects to SP	Chloroquine 25mg/kg over 3 days *	Not approved can be used where resistance to SP is not a problem
3	SP + Amodiaquine	Similar pharmacokinetic profiles	Adverse effects of amodiaquine and SP	Amodiaquine 10mg/kg daily for 3 days *	Approved
4	SP + Quinine	Effective where resistance to SP is not a problem	Drug resistance; Serious adverse effects	Quinine 15mg/kg 12 hourly for 3 days *	Not approved
5	SP+Mefloquin *(Fansimef™)* Each drug has a different pharmacokinetic profile;	Fixed dose pill, single dose	Not an additive or synergistic combination Expensive, Resistance known	Mefloquine 15mg/kg and *	Not approved Not recommended for general use since 1990
6	Atovaquone+Proguanil (Malarone™)	Synergistic activity, Good safety and tolerability in children and adults	High cost Contra-indicated (hypersensitivity or renal insufficiency)	Atovaquone 20mg/kg Proguanil 8mg/kg once daily for 3 days	Approved; Highly efficacious against *P. falciparum.*
7	Chlorproguanil+Dapsone (*LapDap™*)	Well tolerated; Efficacious	Methaemoglobinaemia and haemolysis in G6PD deficiency; Potential cross-resistance with SP	Chlorproguanil 2mg/kg Dapsone 2.5mg/kg once daily for 3 days	Withdrawn by GSK Hemolytic anemia in G6PD deficiency
8	Quinine + Tetracycline	Efficacious	Cinchonism Tetracyclines contraindicated in children and pregnant women Emergence of resistance	Quinine 10mg/kg 8 hourly Tetracycline 4mg/kg 6 hourly for 7 days	Not approved

*= single dose of (SP) Sulfadoxine-pyrimethamine.

4.1.2. Artemisinin-Based Combinations Table (5)

Artemisinin derivatives are particularly effective combination partners because (i) they are very active antimalarials, producing up to 10000-fold reductions in parasite biomass per asexual cycle; (ii) they reduce malaria transmissibility; and (iii) no resistance to these drugs has been reported yet. There are good arguments for those antimalarial drugs that are ineffective alone but using them in combination with artemisinin or one of its derivatives become a new tool for treatment. They, however, cost up to 20 times more than commonly available antimalarials, which is the major limiting factor to quick adoption and implementation of ACTs in developing countries [152]. Artemisinin-based combination treatments (ACTs) are preferred because artemisinin compounds have rapid parasite and fever clearance effects and also reduce gametocyte rate with the potential to reduce transmission [153].

a) Artesunate-Sulfadoxine-Pyrimethamine

Sulfadoxine-pyrimethamine is a fixed combination of a long-acting sulfonamide and the antifolate pyrimethamine. These are synergistic against sensitive parasites. The combination with artesunate is available as separate scored tablets and has been evaluated extensively in adults and children [154-156].

b) Lumefantrine-Artemether

Riamet® has recently been approved as a fixed combination of lumefantrine (benflumethol) (**97**) with artemether, for the treatment of uncomplicated *P. falciparum* malaria [157-159]. There is no accessible clinical data on the efficacy of lumefantrine as a monotherapeutic. Coartemether® is one of the fastest-acting fixed-combination antimalarial with >95% cure rates - even in multi-drug resistant areas [160]. It is used to treat uncomplicated or mixed infections of malaria, caused by the parasite *P. falciparum* and is very well tolerated particularly when compared to the most currently established antimalarials. Moreover it does not show any evidence of organ- or system-specific toxicity, cardiotoxicity, or neurotoxicity [161-164]. Zambia was the first African country to adopt an artemisinin-based combination treatment as its national policy [165].

Table 5. Artemisinin Based Combinations

S. No.	Combinations	Efficacy	Disadvantage	Dose	Status
1	Artesunate+ Chloroquine	Very high chloroquine failure rates (>60%) and sub-optimal efficacy of the combination (<85% cure rate)	---------	---------	Not approved; Not a viable option in areas with pre-existing moderate to high levels of *P. falciparum* resistance to Chloroquine
2	Artesunate+ Amodiaquine	Better efficacy than amodiaquine alone (cure rate >90%); Well tolerated	Neutropenia; Pharmacokinetic mismatch	Artesunate 4mg/kg and amodiaquine 10mg base/ kg once a day 3 days	Approved
3	Artesunate + Mefloquine	In use for many years and the first-line treatment in several parts of SE Asia	Pharmacokinetic mismatch; Mefloquine induced neuropsychiatric effects, cardiotoxic effects, incidents of vomiting in children; but combination with artesunate results in less adverse reactions than the use of mefloquine alone	Artesunate (4mg/kg once daily) for 3 days + mefloquine (25mg base/kg) as a split dose of 15mg/kg on Day 2 and 10mg/kg on Day 3. (Alternatively 8mg/kg mefloquine daily for 3 days)	Not approved
4	Artesunate + Sulfadoxine/Pyrimethamine (SP)	Well tolerated; Efficacy dependent on the level of pre-existing resistance to SP	Pharmacokinetic mismatch; adverse effects to SP	Artesunate 4mg/kg once daily for 3 days and SP single dose of 25mg/kg and 1.25mg/kg respectively	Approved (in areas where SP efficacy is high); Resistance to SP limits the use
5	Artemether + Lumefantrine *(Coartem,™ Riamet™)*	As effective, and better tolerated, as artesunate plus mefloquine; No serious adverse reactions	Irreversible hearing impairment	Artemether 1.5mg/kg Lumifantrine 9mg/kg at 0, 8, 24, 36, 48 and 60 hours	Approved; Not recommended for use in pregnancy and lactating women
6	Chlorproguanil + Dapsone + Artesunate *(Dacart™)*				Withdrawn at development stage by GSK for fears of hemolytic anemia in G6PD deficiency

Table 6. New Combinations (Investigational)

S. No.	Type	Advantages	Disadvantages	Status
1.	Piperaquine+Dihydroartemisinin+Trimethoprim *(Artecom™)* and Artecom™ plus Primaquine *(CV8™)* (CV = China-Viet Nam)	Efficacy consistently above 93%	Animal toxicology studies indicate additive toxicity; No serious adverse events observed in human studies	Trials; May prove to be more affordable
2.	Chlorproguanil + Dapsone + Artesunate *(CDA™ or Lapdap plus™)*	Fixed ratio co-formulation		Trial
3.	Fosmidomycin+ Clindamycin	Both act on the parasite's apicoplast Rapid clearance and 100% cure rates reported		Trials on

Table 7. Antimalarial Vaccines

S. No	Type	Remarks	Target (s)
I	**Pre-erythrocytic Vaccines**		
	1. Circumsporozoite protein (CSP) a) RTS, S: RTS,S/ASO2A, RTS,S/ASO2B, RTS,S/ASO2E b) FP9 ME-TRAP and MVA ME-TRAP ICC-1132 2. CSP + other antigens a) Modified Vaccinia Ankara (MVA) CSP + LSA-1 epitope b) Fowl Pox 9 CSP + LSA-1	Prevent both clinical disease and the transmission of malaria	Sporozoites
II	**Blood-stage/Erythrocytic vaccines**		
	1. Merozoite surface proteins (MSPs) 2. Apical membrane antigen (AMA1 AMA1-C1/FVO 3. Other blood stage antigens i. EBA-175 RII-NG ii. PfEMP1 iii. PfEMP1 DBL1α-TM-AS 4. Combination blood stage vaccines i. PfCP2.9 ii. AMA1-C1/Alhydrogel® plus CPG 7909 iii. MSP3/GLURP (GMZ 2)/AlOH	It prevents invasion of red blood cells by merozoites	✓ Merozoite surface proteins (MSPs) ✓ Apical membrane antigen 1 (AMA1) ✓ The transmembrane erythrocyte binding protein- 175 ✓ Thrombospondin-related anonymous protein (TRAP) ✓ Plasmodium falciparum erythrocyte membrane protein These are surface proteins being targeted
III	**Liver-stage antigens (LSAs):**	Target: T-cell and B-cell level	Liver schizogony and merozoite release
IV	**Sexual-stage vaccines**	In combination with pre-erythrocytic vaccine	Blocks fertilization in the mosquito
V	**Transmission blocking vaccine**	Interruption of malaria transmission from human to mosquito populations	antigens on gametes, zygotes or ookinetes
VI	**Combination Multi-stage Vaccines** 1. FFM ME-TRAP+PEV3A 2. NYVAC-Pf7	Combination of pre-erythrocytic and blood-stage antigens	Multistage of parasite
VII	**DNA vaccine**	Effective in priming the immune responses and inducing immunological memory.	DNA sequence

(Table 7. Contd….)

S. No	Type	Remarks	Target (s)
VIII	**T-cell targeting vaccines**	Could prevent both blood-stage infection and malaria transmission	Production of potent T-cell responses against the liver stage of malaria infection
IX	**GPI (Glycosylphosphatidylinositol) vaccine**	It is a pro-inflammatory endotoxin of parasitic origin	GPI (Glycosylphosphatidylinositol)

4.1.3. New Combinations (Investigational) Table (6)

a) Artecom™

A rapid and powerful plasma schizontocide with rapid symptom control and effectiveness for multi-drug resistant falciparum gametocyte thus minimizing the malaria transmissibility. Six Reasons For Recommending Artecom™: High efficacy, Fast action, Safety, Low recrudescence, Short treatment course, Low drug resistance.

b) CDA™ or Lapdap plus™:

It is a fixed-ratio co-formulation of chlorproguanil-dapsone (Lapdap™) and artesunate. Lapdap™ is entering its final phase of development.

c) Fosmidomycin+ Clindamycin

Fosmidomycin is the first representative of a new class of antimalarial drugs [166]. Clindamycin is another promising drug with a short elimination half-life and a good safety and tolerability profile for antimalarial therapy [167]. The combination of fosmidomycin plus clindamycin (FC) [168, 169] is promising as antimalarial treatment and has a novel and independent mechanism of action.

4.2. Antimalarial Vaccines

Another alternative approach for malaria eradication and/or control is development of a safe, effective and affordable malaria vaccine which might close the lacuna left by other interventions. The ideal vaccine would be safe with no or minimal side-effects, easy and cheap to manufacture, stable for storage/transport, easy to administer, could be given to infants (ideally alongside other childhood vaccinations), would stimulate life-long protection against all forms of the disease. Although extensive work has been done, but it looks like the success is far from the hands of scientists because, maximum number of vaccine candidates failed in their subsequent phases of clinical trials. Being so technologically equipped and having knowledge of DNA sequence of parasite [170], an effective antimalarial vaccine still remains a far-flung vision. Some of the vaccine candidates are shown in Table **7** [171]. Although much effort is directed at disease treatment through the development of new antibiotic drugs, vaccines enjoy many intrinsic advantages. The frequency of vaccine treatment is perhaps, in the range of once or twice per lifetime, to say once or twice per year. Vaccines are relatively cheap to produce if not necessarily to discover, making them of special interest to developing countries.

CONCLUSIONS

Despite the claim by many malaria researchers that novel molecules would provide breakthrough in the treatment of malaria, the factor of resistance still remains the moot question that need to be addressed with urgency. Very few are exhibiting effectiveness against resistant strains among the pool of available molecules. Molecular modeling focusing on mechanism based approach requires more consideration. Novel compounds should not only elicit activity against drug-resistant strains but also meet the criteria necessary for their potency viz. oral bioavailability, stability and affordability. The utilization of natural products seems to be a suitable approach in the search for diverse lead molecules exemplified by artemisinin and its derivatives that are still considered as the touchstone, but limited number of natural products viz. the endoperoxides, lignans and a few from microbial and marine origin have been exploited. Meaningful advances in the development of drug candidates with novel mechanism of action would probably provide new class of effective antimalarials. Research priorities and better participation of pharmaceutical companies in the antimalarial drug discovery and development process is urgently required. Although alternative approaches are successful upto an extent but still they have not left any foot prints.

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