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The current antimalarial therapy includes very few number of potent therapeutic agents. Chloroquine (12) has been the first choice as potent antimalarial therapy but the resistance developed by the parasite declined its clinical use (Wongsrichanalai et al., 2002). Other potent drugs include Primaquine (9) known for the radical cure of malaria (Jain et al., 2004) and Mefloquine (a quinoline methanol derivative of quinine) (White et al., 1999). The combination of chloroquine (12) or amodiaquine (14) with sulfadoxine (21)/pyrimethamine (19) (SP) has been well explored to fight against resistance (McIntosh and Greenwood, 1998).

There has been a consistent effort over this time for the radical cure of malaria. Development of multi-drug resistance, being the most difficult hurdle for success of antimalarial therapy, most of the research is oriented towards overcoming the MDR by one or the other means. Important strategies worked out are multidrug combination, development of derivatives and conjugates of the existing drugs, drug polymer complexes in changing pharmacokinetics, vesicular and particulate delivery for site specificity, approaches to overcome Pgp efflux to achieve desired intracellular drug concentration in parasite cells (Yuan et al., 2008).

2.1 ANTIMALARIAL DRUG DEVELOPMENT

The preface of chloroquine (12) in the 1940s had a marvelous effect on vigor globally; however, the resistance to this drug is very well known today in *P. falciparum* prevailing zones (Wongsrichanalai et al., 2002). The chloroquine (12) resistance has extended all parts of Sub-Saharan Africa.

![Chemical Structure of Chloroquine](image-url)
Consequently, most of the countries changed their first-line antimalarial drugs to sulfadoxine (21) and pyrimethamine (19) combination therapy.

However, resistance to sulfadoxine (21)-pyrimethamine (19) (SP) grew and spread quickly, mainly in South-East Asia and South America (White, N. J., 1992) and in recent times, covering many areas of Africa (Sibley et al., 2001). The worldwide threatening coverage of malarial parasite along with drug resistance creates an urgent need of novel antimalarial agents (Ridley, 2002).

2.1.1 Structure Modification of 4-Aminoquinoline

The antimalarial drug development based on aminoquinoline began with chloroquine (12), which proved to be of greater potency, low toxicity and economic. However, the resistance to this drug by malarial parasite limited its use worldwide. Later on the development of newer analogues such as Amodiaquine (14), were developed which proved to be active against resistant strains of malarial parasite.

However, on account of hepatotoxicity and agranulocytosis due to the toxic metabolite ethyl 2-(1, 4-dihydro-4-oxoquinolin-2-yl)acetate (28), the clinical usefulness of this potent antimalarial candidate was also diminished (Kouznetsov and Gomez, 2009).
2.1.2 Novel Bisquinolines as Antimalarials

The ‘bisquinolines’ based novel antimalarial compounds and their efficacy against chloroquine-resistant strains has been reviewed (Raynes, 1999).

Ayad and co-workers synthesized novel bisquinoline compounds (29-35) and evaluated their activity in both chloroquine-sensitive and resistant strains. The antimalarial efficacy varied as a result of the length of spacer and the compound (35) with n=12 was found to be most potent and better than mefloquine.

Compounds (29) (X=O, n=2) and (30) (X=NH, n=3) are recent examples of the class 4-aminoquinoline units which is linked by 4-position (Ayad et al., 2001).
All three compounds (33), (34) and (35) displayed markedly high activity against the resistant strain. The compound 35 inhibited the decomposition of peroxidation better than 33 and 34.

The results of antimalarial activity of bisquinolines were comparable to that of chloroquine and require further insight into their potential for further development (Ayad et al., 2001).

Several efforts have been accomplished to manufacture and appraise derivatives of primaquine (9) in order to explore the more potent compounds devoid of toxicity.
The development of amino acid based analogues of primaquine provided a considerable augmentation in antimalarial potential (Jain et al., 2004). These derivatives were easily hydrolysable to primaquine regardless of the improved activity/toxicity ratio (Borissova et al., 1995). Further, the covalent coupling of primaquine with lysosomotropic drug carriers—polyacryl starch microparticles have shown significant improvement in antimalarial efficacy of primaquine (9) (Stjarnkvist, 1993). The implants for chloroquine (12) using biodegradable polymers, gelatin and cross-linked gelatin have also been developed. The implants were evaluated for physicochemical properties, in vitro drug release study and pharmacokinetics. The results complied with optimum drug release conforming to the prerequisite of a long term implant for 7 days (Murthy et al., 2001). Their other contribution is reported to the artemether (25) loaded lipid nanoparticles developed by modified thin-film hydration which were found to be safe and more effective with respect to antimalarial potential when compared with standard and marketed formulations (Aditya et al., 2010a).

Examples of therapeutic combinations of mefloquine and artemisinins provide effective treatment of malaria thereby inhibiting the drug resistance in Southern-East Asia (White et al., 1999).

Recently, Capela and associates have designed, synthesized and evaluated the covalently linked hybrids of primaquine (14) and artemisinin (23) which were found to be efficacious at different target sites of malarial parasite (Capela et al., 2011).

### 2.1.3 Isoquinuclidine Analogs of Chloroquine

The various semirigid analogs of chloroquine were synthesized by incorporating isoquinuclidine (2-azabicyclo [2.2.2] octane) ring system (36-41) in numerous pharmacophores to get better pharmacological activity. The analogs were tested in vitro against *Plasmodium falciparum* strains and *Leishmania donovani* promastigote cultures. Compounds 36 and 39 [R=CH₂N(CH₃)₂] showed antimalarial activity against both sensitive and resistant strains. (Khan et al., 2007).
2.1.4 Novel Chloroquine and Thiazolidenone Scaffolds as Antimalarials

The thiazolidin-4-ones have been explored to be remarkable scaffold for the design and synthesis of novel chloroquine based hybrids (Madrid et al., 2006). Ruiz and co-workers, 2011 synthesized a series of compounds and those with benzyl amino fragment, showed 3-fold antimalarial potency than chloroquine.

The analogues based on 4-benzylamino-7-chloroquinolines (42-45) exhibited better antimalarial activity than hybrid analogues (46-62).
Synthesis and Studies on Polymer-linked Combined Antimalarial Drugs To Overcome The Emergence of Drug Resistance For Radical Cure of Malaria
Compounds 42, 57 and 61 showed marvelous antimalarial efficacy with low toxicity (Ruiz et al., 2011; Solomon et al., 2007).
2.1.5 4-Aminoquinoline Analogs

A short side-chain derivative of 4-aminoquinoline AQ-13, (63) which is analogue of Chloroquine was synthesized and currently entered Phase I clinical trials after outstanding biological results (O’Neill et al., 2012).

![Chemical Structure](image)

(63)

2.1.6 Trioxaquine Derivatives

SAR116242 (PA1103), (64) also called Trioxaquine, led to 100% parasitemia reduction on administration of 32 mg (70 µmol)/kg on day 4 in humanized mice infected with *P. falciparum*. The *cis* and *trans* isomers were found to be equipotent against the FcM29 *P. falciparum* strain (IC$_{50}$ = 12 and 8 nM, respectively) (Cosledan et al., 2008).

![Chemical Structure](image)

(64)

2.1.7 Novel Isoquines Derivatives

The isoquine based analogue (GSK369796) (65) bears *tert*-butylamino moiety, by virtue of which the compound shows improved metabolic stability. IC$_{50}$ values against Chloroquine-resistant strains range from 11 to 17 nM. In *P. berghei* infected mice an ED$_{50}$ of 2.8 mg (7.8 µmol)/kg is obtained (O’Neill et al., 2009).
2.1.8 Novel Tetroxane Derivatives as Antimalarials

The tetroxane derivative [RKA 182], 66 is synthetic peroxide. Heme alkylation has been suggested as its mode of action. IC$_{50}$ values against 3D7 and K1 strains were below 1 nM in *P. berghei*-infected mice, it showed ED$_{50}$/ED$_{90}$ values of 1.33/4.18 mg (1.6/4.9 µmol)/kg. With the formation of the ditosylate salt, an increased oral bioavailability in rat (38%) and mouse (42%) models was achieved (O’Neill et al., 2010).

2.1.9 Cinnamic acid and 4-Aminoquinoline Conjugates

A series of cinnamic acid and 4-aminoquinoline conjugates was synthesized through dipeptide linkage as potential antimalarials with dual mode of actions. Among the synthesized analogues 67 was found to be most potent antiplasmodial agent inhibiting hemozoin formation and enzyme catalytic Cys residues and thus proved to be promising lead molecules for the development of new antimalarials (Perez et al., 2012).
2.1.10 Structure Modification of 8-Aminoquinoline

A series of novel bis(8-aminoquinolines) (68-74) using expedient one to four steps synthetic procedures was synthesized. Selected compounds 68, 73 and 74 exhibited remarkable antimalarial potency (Kaur, et al., 2011). The plasmodial LDH (lactate dehydrogenase) assay was performed for evaluation of in vitro antimalarial efficacy (Makler and Hinrichs, 1993).

Of these analogues, 69, 70 and 71 were found to be most potent when compared with the pentamidine as standard drug, (72).

(69) \( R=OC_5H_{11}, R_1=C_2H_5, R_2=H, X=CO \)
(70) \( R=H, R_1=H, R_2=C(CH_3)_3, \)
(71) \( R=H, R_1=H, R_2=H, X=CH_2CH_2NHCH_2CH_2 \)
2.1.11 Novel NPC 1161 B as Antimalarial

NPC 1161 B, (75) is the (-)-enantiomer of the racemic 8-aminoquinoline, NPC 1161 C (Dutta et al., 2011) which exhibited significant activity against *P. berghei* infected mice. After treatment with 4 mg (9 µmol)/kg/day thrice all animals were cleared of parasites at day 6 and found to be alive at day 34 (Chesney et al., 2008).
2.1.12 Novel Naphthyridine Analogues

A novel series of naphthyridine based potent antimalarial drugs was developed and tested for biological activity. All compounds (76-87) showed better *in vitro* results than chloroquine against *P. falciparum* (Zhu et al., 2007; Desjardins et al., 1979; Chulay et al., 1983).

![Chemical structures of novel naphthyridine analogues](image)
All compounds (76-87) revealed remarkable antimalarial activity and less toxicity against both *P. berghei* and *P. yoelii* nigeriensis infected animals. The replacement of methyl group in compound 76 by isopropyl group as in 78 improves the biological activity six times.

Some of these compounds (78, 81, 84, and 87 where R=CH(CH₃)₂) exhibited ten times better antimalarial activity than chloroquine. Thus, these molecules prove to be potential candidates for further development of newer antimalarial drugs (Zhu et al., 2007).

### 2.1.13 Tafenoquine as Antimalarial

Tafenoquine (88) was synthesized based on pharmacophore of primaquine for the total elimination of *P. vivax*. The molecule was potent but displayed vortex keratopathy as major side effect in clinical trials but this issue was sorted out soon. (Nasveld et al., 2010). In further studies Leary and co-workers observed no such adverse effects (Leary et al., 2009).

![Tafenoquine molecule](image)

### 2.1.14 Novel Artemisinin Analogs as Antimalarials

The drug resistance to arsenol has been limiting its clinical utility (Valderramos and Fidock, 2006; Ekland and Fidock, 2008; Nkrumah et al., 2009; Sidhu et al., 2002; Sidhu et al., 2005; Sidhu et al., 2006). Therefore, artemisinin-based combination therapies (ACTs) became the first choice for the treatment of *P. falciparum* malaria worldwide (Eastman and Fidock, 2009). However, a decline in the
efficacy of ACTs has been observed on the border of Thailand and Cambodia (Dondorp et al., 2009; Lim et al., 2009; White, 2008). The development of resistance to artimisinin derivatives puts forth dire need for the invention of novel antimalarial drugs.

Nagelschmitz and co-workers synthesised artemisone, (89) which was found to be a highly potent and safe molecule devoid of any toxic effect in phase I clinical trials (Nagelschmitz et al., 2008).

![Image of artemisone molecule](image)

Its IC$_{50}$ values during in vitro studies are 1.49 nM (K1 strain) and 1.59 nM (TM90-C2A strain) (Nagelschmitz et al., 2008). Further its efficacy has also been indicated against cerebral malaria in *P. berghei* infected animals (Grinberg et al., 2010).

### 2.1.15 Novel Indoloquinoline Derivatives as Antimalarial Drugs

Wright and associates have synthesized a series of indoloquinoline based analogues (90-92), out of which 2, 7-dibromocryptolepine (92) was found to be nine times more potent than chloroquine with no toxicity (Wright et al., 2001). These analogues show antimalarial activity by inhibition of haemozoin formation (Onyeibor et al., 2005). The compounds 90 and 91 are currently under investigation for the development of newer antimalarial agents.
2.2 RECENT TRENDS IN ANTIMALARIAL DRUG DEVELOPMENT

2.2.1 Indole Alkaloid Derivatives

Novel indole alkaloids derivatives 93 and 94 were tested orally and subcutaneously in *P. berghei* infected murine model provided 100% protection with mean survival time more than 40 days for the treatment of malaria (Rocha *et al.*, 2012).

2.2.2 Novel Dihydroartemisinin-Nitric oxide Donor Hybrid Derivatives

Novel dihydroartemisinin-nitric oxide donor hybrid based derivatives have been designed and synthesized as multitarget agents. These hybrids have been evaluated for the treatment of cerebral malaria against *P. berghei* (ANKA). The hybrid 95 has been proved to be potential antimalarial drug candidate with improvement of survival rate (Bertinaria *et al.*, 2015).
2.2.3 Novel Quinazolin-4-one Derivatives

Novel glycine coupled sulfonamide at 2-position in 4-quinazolin-(3H)-ones have been synthesized and found to be dihydrofolate reductase inhibitors as antiplasmodial compounds. The most active compounds 96 and 97 were studied further for inhibitory activity against h-DHFR and Pf-DHFR receptors by in silico methods and also exhibited good bioavailability after ADME studies (Patel et al., 2015).

![Chemical structures](image)

(96) (97)

2.2.4 Novel Tambjamines and Prodiginines Derivatives

Novel bipyrrrole and tripyrrrole derivatives as tambjamines and prodiginines have been discovered as first antimalarials of this class in multidrug-resistant *P. yoelii* infected mice.

![Chemical structure](image)

(98)

Among the synthesized series of tambjamines and prodiginines, the compound 98 provided 100% protection to the infected mice (Kancharla et al., 2015).
2.2.5 Novel 1,2,4-Trioxepanes Substituted Derivatives of D-Glucose

Novel 1,2,4-trioxepanes substituted derivatives of D-glucose have been synthesized and tested \textit{in vitro} against W2 chloroquine-resistant \textit{P. falciparum}.

![Chemical structure](image)

The compound 99 was found to be the most potent among the synthesized derivatives in low mM concentration (Sonawane \textit{et al.}, 2015).

2.2.6 Novel Benzoxaborole Derivatives

Novel benzoxaborole derivatives have been designed, synthesized and evaluated for antimalarial potential. The compound 100 exhibited remarkable \textit{in vivo} antimalarial efficacy in \textit{P. berghei} infected mice (Zhang \textit{et al.}, 2015).

![Chemical structure](image)

2.3 POLYMER-DRUG CONJUGATES IN MALARIA

2.3.1 Primaquine – Glucosamine Conjugates

Novel conjugates of primaquine and glucosamine (101-103) employing polyaspartamide type polymeric materials were designed, synthesized and evaluated for antimalarial activity in \textit{P. berghei} infected animals. The incorporation of polymeric carriers further lead to the potentiation of antimalarial efficacy as
compared to glucosamine conjugates devoid of polymers (Raji et al., 2009).

\[
\text{Primaquine-glucosamine conjugate (101)}
\]

\[
(102a) \text{ (PHEA-PQ) } Z = O \\
(102b) \text{ (PHEA-PQ) } Z = O \\
(103) \text{ (PHPA-PQ) } Z = \text{CH}_2
\]

\[
\alpha \text{ unit } X = \text{CH}_2, \ Y = O \\
\beta \text{ unit } X = O, \ Y = \text{CH}_2
\]

2.3.2 Prodrug of Glutathione Reductase Inhibitor Conjugated with a 4-Aminoquinoline

Novel prodrugs based on 4-aminoquinoline conjugated with glutathione reductase (GR) inhibitor were designed, synthesized and evaluated against resistant strains of *P. falciparum*. The prodrug 104 exhibited remarkable antimalarial
potentiation prolonging the mean survival time of treated animals with no toxic effects (Charvet et al., 2001)

![Chemical Structure](image1.png)

(104)

### 2.4 COMBINATION THERAPY AS ANTIMALARIALS

A number of strategies have been employed to fight against drug resistance for effective treatment of malaria. The combination based drug regimens have been reported e.g. chloroquine/amodiaquine with sulfadoxine/pyrimethamine (SP) and exhibited potentiation of antimalarial activity (McIntosh et al., 1998). Currently, artemisinin based combination therapy is being employed, one of them includes artemisinins and mefloquine which provide effective results in the treatment of *P. falciparum* resistant malaria (White et al., 1999).

Novel combination therapy based hybrids involving 4-aminoquinoline part of chloroquine (12) with clotrimazole (105) have been explored as potential antimalarials. The hybrid compound 106 was found to be most suitable for chloroquine-resistant parasites with optimum half-life (Gemma et al., 2012).

![Chemical Structure](image2.png)

(105) (106)
A novel antimalarial combination regimen of chloroquine (12) + azithromycin (107) + R-enantiomer of amlodipine (108) (R-enantiomer was selected because of racemic mixture being cardiotoxic), provided up to 99.9% suppression of parasitemia in *P. yoelii* infected murine model. This combination provided synergistic antiplasmodial efficacy with good safety profiles (Pereira *et al.*, 2011).

![Chemical structures](107.png)

A novel combined tablet dosage form of artesunate (24) + amodiaquine (14) with fixed dose has been designed and formulated for the treatment of drug-resistant *P. falciparum* malaria showing a remarkable efficacy resulting in 100% parasitological response rate. The drug regimen was found to be safe and well tolerated (Sinou *et al.*, 2009).

A new combination of curcumin (109) + artemisinin (23) was tested on a clone of *P. chabaudi* for the treatment of drug-resistant malaria. The piperine was added to enhance the activity and the results revealed a moderate antimalarial efficacy of the combination (Martinelli *et al.*, 2008).

![Chemical structure](109.png)
In North America, the combination of atovaquone (110) + proguanil (17) provided more than 93% curative and prophylactic protection against chloroquine and multi-drug resistant *P. falciparum* malaria with a remarkable safety profile (Boggild *et al.*, 2007).

![Chemical Structure of Atovaquone (110)](image1)

The WHO recommended the most recent combination therapy for malaria including dihydroartemisinin (26) + piperaquine (111) which was investigated for the development of resistance by malarial parasite on account of K13 mutant allele in western Cambodia. Therefore, new artemisinin based combination therapies need to be explored (Leang *et al.*, 2015).

![Chemical Structure of Piperaquine (111)](image2)

A comparative analysis of different combination regimens of artemisinin (23) + naphthoquine (112), artemether (25) + lumefantrine (6), dihydroartemisinin (26) + piperaquine (106) and artemether (25) + chloroquine (12) in *P. berghei* infected mice, revealed that combination of artemisinin (23) + naphthoquine (113) for three successive days at dose of 7.3 mg/kg provided significant parasite elimination in
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comparison to single dose with a higher mean survival rate (Sunday and George, 2013).

![Chemical structure]

(112)

2.5 PHARMACEUTICAL FORMULATIONS AND RELATED APPROACHES

The development of macromolecular prodrugs is advantageous in terms of controlled and targeted drug delivery (Duncan, 2002). Several other approaches such as ethyl cellulose-based transdermal therapeutic system (Mayorga et al., 1997) colloidal carrier liposomes, polymeric and lipid nanoparticles (Date et al., 2007) and artificial chylomicron emulsion etc. are well established drug delivery systems (Dierling and Cui, 2005).

The process of multi-drug resistance MDR is believed to involve over-expression of P-glycoprotein (Yuan et al., 2008). Co administration of MDR1-related chemotherapeutic drugs with an MDR modulator may enhance the bioavailability of these agents sufficiently to enable oral dosing, which would potentially be more convenient and less toxic (Sikic, 1999). The strategies to overcome p-glycoprotein mediated MDR resistance of anticancer drugs particularly by using particulate drug carriers have been reviewed (Shah and Murthy, 2007).

The drug targeting to liver stage during malaria in amino acid sequences of *P. berghei* has been accomplished through liposomal nanovessels (Robertson et al., 2008). Similarly, artemisinin loaded PEGylated liposomes have been explored as
effective nanocarrier systems to provide long term exposure of the drug concentration to parasites. (Isacchi et al., 2011)

The nanoparticles drug delivery system play a vital role in treatment of malaria because of long term availability of the drug at the site of action achieving the targeted drug delivery with improved efficacy and minimum toxicity (Mosqueira et al., 2004).

The most recent polymeric drug delivery systems such as nanoaggregates e.g. polyamidoamine based polymers have also been explored for targeting infected RBCs during malaria for the targeted drug delivery (Urban et al., 2014).

Moreover, 2,2-bis(hydroxymethyl) propionic acid (bis-MPA) monomers based dendrimers loaded with a combination regimen of chloroquine and primaquine has been found to be beneficial for the patients with glucose-6-phosphate dehydrogenase (G6PD) deficiency thereby eliminating haemolysis caused by primaquine when given alone (Movellan et al., 2014). Thus, nanotechnology plays a critical role for the treatment of drug-resistant malaria by achieving targeted drug delivery to the malarial parasite (Santos and Mosqueira, 2010).

A series of artemisinin derivatives (113-117) in a gel formulation and assessed for their antimalarial activities in addition to artelinic acid, which was demonstrated previously to possess good prophylactic as well as curative antimalarial activity against Plasmodium berghei by transdermal administration were synthesized. Artemisinin (23) the parent compound of the series, showed moderate prophylactic but poor curative activity. Although methyl artelinate (115) was more active against P. berghei than artelinic acid (113) and sodium artelinate (114) by subcutaneous injection, its transdermal curative and prophylactic activity was only comparable with or weaker than that of artelinic acid (113).
Conversely, both dihydroartemisinin trimethylsilyl ether (116) and dehydrodihydroartemisinin (117) showed weaker antimalarial activity than artelinic acid by the subcutaneous route, yet exhibited comparable activity by transdermal administration. Artemether (25), a prodrug of dihydroartemisinin (26), is as effective as the parent dihydroartemisinin (26), both compounds were the most potent agents among the compounds studied, with total prophylactic and curative doses of 30 mg/kg and 60 mg/kg, respectively. Complete absorption of dihydroartemisinin (26) appears to occur within 5 min. after application. In general, authors found that the prophylactic dose is about half that of the curative dose under the protocols used in this study. This novel drug delivery system may be an easy and safe way to administer artemisinin-type antimalarials and also a good alternative dosage form for active compounds with solubility problems (Lin et al., 1994).
2.6 MISCELLANEOUS APPROACHES

The emergence of drug resistance is the major limitation of the currently available antimalarial agents. Thus, there is an urgent need for the development of novel approaches for the treatment of drug-resistant malaria. Some of the novel and emerging areas have been explored for the development of new antimalarial therapy and are discussed below:

2.6.1 RNA interference (RNAi)

RNA interference (RNAi) is a technique of interfering with gene expression causing degradation of mRNA which finally leads to decrease in protein synthesis (Fire, 1999). The results of a recent study reveal that the addition of PfUDN dsRNA to the culture of *P. falciparum* inhibited the growth of parasite during early growing stages of the parasite (Tarique *et al.*, 2014). The down-regulation of hypusinated eIF-5A and DHS has been observed when culture of *P. berghei* ANKA merozoites was transfected with eIF-5A-shRNA and DHS-shRNA, respectively (Schwentke *et al.*, 2012). The RNAi technique is beneficial for the study of gene function in *P. falciparum* as it is a short duration procedure, economic and the simultaneous analysis of multiple number of genes can be performed conveniently. However, it requires electroporation efficiency and marker phenotype which are yet to be explored further (Zhang *et al.*, 2014).

2.6.2 Stem cells

Recently, atypical progenitor cells have been identified in the mice infected with malaria which give rise to a large number of cells having potential to combat the drug-resistant malaria (Belyaev *et al.*, 2010). When the stem cells were transplanted to the mice infected with complicated malaria, this therapy proved to be a boon in the treatment of malaria (Saei and Ahmadian, 2009).

Recently, mesenchymal stem cells (MSCs) based therapy cured the *P. berghei* infected mice by altering Treg-cell responses and provided an effective treatment for
the malaria (Thakur et al., 2013). Further, haematopoietic stem cells were also found to be an efficient approach for the treatment of *P. berghei* infected mice to fight against malaria (Asami et al., 1991). It is anticipated that the stem cell therapy will become an alternative approach for the treatment of the drug-resistant malaria in the near future based on the results of the research scenario in this particular thrust area (Zhang et al., 2014).

### 2.6.3 Peptides

Among the various approaches for the treatment of drug-resistant malaria, the peptides are also reported to have antimalarial potential which can either be obtained from natural resources like plant, fungi and bacteria or developed by synthetic origin (Krugliak et al., 2000). For example, tyrothricin obtained from bacterium *Bacillus brevis* inhibits parasite growth by lysing the infected RBCs for the treatment of malaria (Rautenbach et al., 2007). Another peptide antiamoebin I has been obtained from the fungus *Emericellopsis poonensis* which interferes with functions of mitochondria of the parasite (Nagaraj et al., 2001). The peptide developed by synthetic route is benzyloxycarbonyl Z-Phe-Arg-CH2F which acts on cysteine proteinase in *P. falciparum* and inhibits degradation of haemoglobin (Rosenthal et al., 1991).

### 2.7 Antimalarial Drugs in Clinical Trials

In order to achieve the radical cure of malaria, there is need to develop the multistage targeting drug delivery systems. However, many drugs and vaccines have reached the different phases of clinical trials and targeting the different stages of life cycle of malarial parasite. For example, the malaria vaccine 257049 is now in Phase III of the clinical trials which targets both liver and blood stages of the plasmodium. Similarly, an imidazolopiperazine; GNF156 has entered the phase II of the clinical trials and found to be effective in all stages of malarial parasite (Sinha et al., 2014).
The candidate has carried out research on the project entitled, “Synthesis and Studies on Polymer-linked Combined Antimalarial Drugs To Overcome The Emergence of Drug Resistance For Radical Cure of Malaria.” The polymer used in the investigation pertains to polyphosphazenes, therefore, it was considered of interest to give a brief account of polyphosphazenes next.

### 2.8 POLYPHOSPHAZENES

Polyphosphazenes are inorganic polymers containing nitrogen and phosphorus with chlorine atoms on polymeric backbone. These are the most versatile inorganic polymers because a wide variety of substituents can be attached to the backbone phosphorus atom to give a wide range of stable poly(organophosphazenes) (Figure 2) which result in a very broad spectrum of physical as well as chemical properties suitable for many potential applications specifically biomedical and polymeric drug delivery systems (Allcock, H. R., 1995; Allcock H. R., 2003; Potin and Jeager, 1991).

![General structure of Poly(organophosphazenes)](image)

**Figure 2: General structure of Poly(organophosphazenes)**

Bhardwaj and co-workers have prepared several substituted polyphosphazenes having diversified physicochemical properties to have site specific drug delivery of anticancer drugs to the colon (Sharma et al., 2014; Singla et al., 2014).

The trimer hexachlorocyclotriphosphazene (118) has been obtained by the reaction of ammonium chloride with phosphorus pentachloride yielding a mixture of cyclic and linear phosphazene and found to be the most suitable route for the synthesis of hexachlorocyclotriphosphazene required for further polymerization.
The trimer, (hexachlorocyclotriphosphazene) (118) can be sublimed at 50 °C under reduced pressure, and is soluble in organic solvents like tetrahydrofuran which was further utilized for the synthesis of substituted cyclophosphazenes (Lund et al., 1960; Emsley and Udy, 1971; Allcock et al., 1987).

Figure 3: Reaction scheme of Polyphosphazene

The most remarkable progress in the area of reactive and functional polymers from the chloronitrides of phosphorus (II) was manifested by Stokes (Stokes, 1897). This first attempt to polymerize the compound by heating at high temperature for a long time resulted in an insoluble, inflexible and useless elastomer know as “inorganic rubber”. Thereafter, the trimer hexachlorocyclotriphosphazene (118) was heated in pyrex ampoules made from glass tubes, under vacuum at 250±5°C for specified time and the visual examination of the viscosity of the reaction indicated the progress of the polymerization and gave information about its end product (Figure 3) (Devadoss and Nair, 1982).
The prolonged heating or employing higher polymerization temperature result in the formation of the cross-linked material referred to as inorganic rubber (Stokes, 1897).

2.8.1 Reactivity of Poly(dichlorophosphazene)

The high reactivity of the polar P-Cl bond makes poly (dichlorophosphazene) a functional polymer on account of chloride replacement, without P=N bond cleavage, by different organic compounds to synthesize poly (organophosphazene) useful for many applications (Vandrobe et al., 1997) (Figure 4).

The various cyclo(organophosphazenes having diversified physicochemical properties have been usually prepared by nucleophilic substitution of the reactive chlorine of hexachlorocyclotriphosphazene (118) with different aliphatic and aromatic alcohols, aliphatic and aromatic amines & thioorganic derivatives are shown in Figure 4 (Alcock, 1967; Alcock, 1972; Shaw, 1978; Carroll and Shaw, 1962; Carroll and Shaw, 1966).

Figure 4: Reactivity of Hexachlorocyclotriphosphazene with various reagents
The physicochemical properties of the substituted polyphosphazenes have been dependent on the substituents attached to it and not on the polymeric backbone. Therefore, a wide variety of substituted poly(organo) phosphazenes could be synthesized by making appropriate choice of the substituents to have the desired physicochemical properties e.g. bioinertness, hydrophobic, hydrophilic properties and biodegradability characteristics of the polymeric materials.

The biodegradable polyphosphazenes synthesized by variation in the side chains determines their applications in the area of drug delivery (Ottenbrite et al., 1992; Siepmanna and Gopferichb, 2001).

2.8.1.1 Substituents for Bioinertness

The polyphosphazenes of interest as bioinert materials include strongly hydrophobic and hydrophilic materials. The physicochemical properties of the substituted polyphosphazenes vary from water soluble (120) (Luten et al., 2008) to the superhydrophobic (121) (Allcock et al., 2006b) (Figure 5).

\begin{align*}
\text{(120)} & \quad \text{(121)}
\end{align*}

Figure 5: Poly(organophosphazenes) with versatile properties

This has given versatile properties of poly(organophosphazenes) and resulted in a variety of materials suitable for biomedical applications (Alcock H. R., 2006a). A combination of identification and release sites (Teasdale et al., 2011), amphiphilic polymers (Zhang et al., 2006) and solubility enhancing moieties (Carriedo et al., 2004) have also been well explored.
2.8.1.2 Hydrophobic Substituents

Aryloxyphosphazene polymers such as compound 122 and hybrid organosilicon-organophosphazene polymer 123 are also hydrophobic and find applications as biomaterials (Allcock and Connolly, 1985).

\[
\left[ \begin{array}{c} \text{N} & \text{P} \\ \text{OC}_6\text{H}_5 & \text{OC}_6\text{H}_5 \end{array} \right]_n
\]

(122)

\[
\left[ \begin{array}{c} \text{N} & \text{P} \\ \text{OC}_6\text{H}_5 & \text{OC}_6\text{H}_5 \end{array} \right]_n
\]

(123)

2.8.1.3 Hydrophilic Substituents

There are many hydrophilic polyphosphazenes with alkylamine (-NHCH₃, -NHC₂H₅ etc.) ether, and hydroxyl/carboxyl bearing functional groups. (Allcock et al., 1986; Allcock et al., 1992). The substitution of polyphosphazenes with both hydrophilic and hydrophobic moieties provides a wide range of polyphosphazenes with different applications (Vandrobe et al., 1997).

2.8.1.4 Insoluble Biodegradable Polyphosphazenes

Polymers of this type are candidates for use as erodible biostructural materials, sutures, or as matrices for the controlled delivery of drugs. The following examples explain the scope of such biodegradable polyphosphazenes.

2.8.1.5 Amino Acid Ester Substituted Polyphosphazenes

The polyphosphazenes substituted with amino groups degrade by hydrolysis (Austin et al., 1983). Therefore, such polymers have been employed for the formulation of drug loaded particles (Goedemoed, 1990) Figure 6.
Several mechanisms have been proposed for the hydrolytic degradation of polyphosphazenes (Allcock et al., 1994).

### 2.8.1.6 Steroidal and Amino Acid Ester Substituted Polyphosphazenes

Steroids such as 124 and 125 have been linked to a polyphosphazene chain by sodium salt of the hydroxyl group employing amino acids esters and other co-substituents (Allcock and Fuller, 1980).
2.8.1.7 Imidazolyl Substituted Polyphosphazene Derivatives

The imidazolyl substituted polyphosphazenes are hydrolysable polymers such as 126 (Allcock and Fuller, 1981). The rate of hydrolysis can be altered by hydrophobic co-substituents as in 127 (Laurencin et al., 1987).

\[
\begin{align*}
(126) & \\
(127) & 
\end{align*}
\]

2.8.1.8 Schiff’s Base Substituted Polyphosphazenes as Antibacterial Agents

Many drugs having antibacterial property like sulphadiazene have been linked with polyphosphazenes through aldehydic group 128 which can form Schiff’s base substituted polyphosphazenes (129). The active drug is released in its molecular form after the cleavage of hydrolytically unstable Schiff’s base linkage in aqueous media (Allcock and Cheg, 1989).

\[
\begin{align*}
(128) & \\
(129) & 
\end{align*}
\]
2.8.1.9 Carboxylic Acid Substituted Polyphosphazenes through Amide Linkages

The carboxylic acid functional groups containing bioactive molecules, such as polypeptide, N-acetyl-DL-penicillamine, \( p \)-\((dipropyl-sulphamoyl)\) benzoic acid have been conjugated with polyphosphazene chain. The reduction of polyphosphazenes that bear \( p \)-cyanophenoxy side group alongwith phenoxy cosubstituents yielded the polymer (130). Further, the dicyclohexyl-carbodiimide induced condensation with the carboxylic acids gave polymers of type 131 (Allcock et al., 1982)

\[
R = \begin{array}{c}
\text{SO}_2\text{NH} \\
\text{N}
\end{array}
\]

The process of polymer degradation results into small monomers after cleavage of polymeric backbone (Gopferich and Tessmar, 2002). The polymer bulk or backbone undergoes the process of erosion leading to material loss (Nair and
Laurencin, 2007). The erosion process is prompted by degradation and is a matter of hydrolysis only, e.g. polyanhydrides need to undergo degradation prior to erosion (El-Amin et al., 2006). Polyphosphazenes can undergo hydrolytic degradation by surface and bulk erosion (Laurencin et al., 1992).

Recently some controlled molecular weight polyphosphazenes with narrow polydispersities have been synthesized by cationic polymerization (Teasdale et al., 2011). The photoactive drug hypericin conjugated with polyphosphazene have been synthesized by living polymerization method. These polymers were found to be suitable for photodynamic therapy (Teasdale et al., 2012). Similarly pegylated polyphosphazenes bearing isoleucine ethyl ester groups were employed for the sustained release of the anticancer drugs 5-fluorouracil (Lee et al., 2009) and doxorubicin (Kang et al., 2005). The hydrogels of aqueous solution of poly[(organo)phosphazene] containing doxorubicin were prepared at human body temperature (Kang et al., 2006; Chun et al., 2009a). The bioavailability of the hydrophobic drug silibinin was enhanced by using similar polymers (Cho et al., 2012), which exhibited significant antitumor effect on account of the sustained release of the drug. When multiple thiol groups were added as different substituents to polyphosphazenes, this approach enhanced the gel strength (Potta et al., 2009).

2.8.2 Substituted Polyphosphazenes for Vaccination

The substituted polyphosphazenes plays an important role in the delivery of vaccine as an emerging polymer of the era. The role of substituted polyphosphazenes for influenza vaccine was first investigated. The mechanism of action involves the enhancement of the immune response to all strains of HA (hemaggulatination) influenza and assayed by HAI (hemaggulatination inhibition) which was found to be ten folds more potent than the level of vaccine alone (Payne et al., 1998). Recently, a potent adjuvant polyphosphazene
derivative named poly[di(sodium carboxylatoethylphenoxy)phosphazene] (PCEP) has been explored for its immunization potential in mice model (Mutwiri et al., 2007). It also enhanced antigen-specific immune response to HBsAg in mice (Mutwiri et al., 2007). Further, some polyphosphazenes have been developed as the potent interdermal immunoadjuvants after evaluation in pigs using recombinant hepatitis B virus surface antigen (HBsAg). (Alarcon et al., 2007; Kenney et al., 2004; Prausnitz, 2004; Andrianov et al., 2005).

2.8.3 Polyphosphazenes in Tissue Engineering

The role of polyphosphazene in tissue engineering due to their synthetic flexibility was first reported by Laurencin and co-workers (Laurencin et al., 1992). Later on the bone scaffolding applications were explored (Nair et al., 2006). Further, polyphosphazene have been found to be potential candidate for bone tissue engineering after in vitro and in vivo osteocompatibility studies (Sethuraman et al., 2006). The glass transition temperature (Tg) has been found to be an essential and determining factor for a polymer to be used as material for bone regeneration which must be higher than physiological temperature of the body. The bulkier group such as phenyl- alanine substituted polyphosphazene having higher Tg (42°C) are found to be suitable for bone tissue engineering (Nukavarapu et al., 2008).

2.8.4 Polyphosphazene-Drug Conjugates: Thermosensitive Hydrogels

A wide range of thermosensitive hydrogels of polyphosphazenes substituted with hydrophilic, hydrophobic and other substitutions on the polymeric backbone have been designed and synthesized with enhanced biocompatibility and non-toxic degraded products (Lee et al., 1999a, 2002, 2003, Lee and Song, 2004). Many anticancer drugs like doxorubicin and paclitaxel
have been conjugated with polypophosphazene and formulated to hydrogel system for the controlled drug delivery in which the L-isoleucine ethyl ester (IIeOEt) as hydrophobic group and α-amino-ω-methoxy-poly(ethylene glycol) as hydrophilic group alongwith carboxylic acid as a functional group were employed as substituents. Further, in vivo results have shown that the developed hydrogel system suppressed the tumour growth more effectively with less toxicity for prolonged time after local injection at the tumour site when compared with doxorubicin and paclitaxel as plain standard drugs (Chun et al., 2009a, 2009b).