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Review

4-Aminoquinoline-hybridization *en route* towards the development of rationally designed antimalarial agents

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Abstract: Resistance of *Plasmodium falciparum*, the causative agent of malaria, against quinine and chloroquine along with the lack of malaria vaccines has encouraged the development of various synthetic strategies towards biologically active scaffolds. An emerging strategy in medicinal chemistry, termed as molecular hybridization, involving the covalent fusion of two or more drugs, active compounds, and/or pharmacophoric units in to a hybrid compound, with fascinating activities and multiple but not essentially simultaneous pharmacological targets. 4-aminoquinolines are considered as previleged antimalarials and 4-aminoquinoline hybridization is considered as an attractive and feasible approach for the development of new molecular frameworks for averting and delaying the emergence of drug resistance along with improvement in efficacy. The present review article describes the recent developments on the 4-aminoquinoline-hybridization towards the development of new antimalarials.

1. Introduction

In 20th century, the drug design approach accomplished "one-target-one-drug" concept for the discovery of new drugs which, without any iota of doubts, stay dominant for many years. According to this concept, single drug is designed for a single target. Although, numerous drugs have been designed and used clinically with good success using this approach, yet the strategy of one drug hammering one target remains inadequate for the treatment of several diseases such as neurodegenerative syndromes, cardiovascular diseases, diabetes and cancer which involve multiple pathogenic factors.[1] Further, an ideal drug for one target does not always have clinical efficacy either due to non-recognition of *in vivo* target or the inability to access the site of action. This target-based strategy does not always guarantee success since some selective drugs can work only in selected number of patients. For example, Astra Zeneca's Iressa (gefitinib) is designed to treat lung cancer *via* targeting EGFR (Epidermal Growth Factor Receptor) protein. The drug provided an extremely potent response, but only in case of about 10% of the infected individuals.[2,3] The ineffectiveness of single medicine paradigm necessitated the need to discover new paradigms where the drug therapy can block more than one targets.

Among the different strategies developed to address the above issues, combination therapy was considered as a popular alternate in which cocktail of drugs are co-administered in form of two or more individual tablets to treat unresponsive patients.[4] However, the advantage of combining therapeutic mechanisms of different drugs by this approach is compromised with patient compliance.[5,6] The multi-component drug approach, involving co-formulation of two or more drugs in a single tablet, made dosing regimens simpler, improved patient compliance[7,8] and even obviated the risk of drug-drug interactions present in combination therapy. This strategy has enhanced the Research and Development (R&D) as evident by the launch of several multi-component drugs which includes Caduet[9] (atorvastatin + amlodipine) and Vytorin[10] (simvastatin +ezetimibe) for treatment of cardiovascular disease, Coartem (artemether+lumefantrine) and Artekin (dihydroartemisinin+piperaquine) for the treatment of malaria[11] and Atripla[12] (efavirenz+tenofovir/emtricitabine) against AIDS. However, highly complex Pharmacokinetic (PK)/Pharmacodynamic (PD) relationships in multi-component drugs due to the differences in relative rates of metabolism of drugs in different patients, led to unpredictable variability. With the failure of these strategies, the development of new drug molecules that aim to modulate multiple targets simultaneously (polypharmacology) along with enhanced efficacy, improved safety, synthetic selectivity and economic accessibility represented a big challenge for pharmaceutical sector.

Now a days, molecular hybridization has successfully emerged as a promising tool for medicinal chemists and drug design process, in which two or more different pharmacophoric units are covalently linked into a single hybrid molecule with superior affinity and efficacy as compared to the parent drugs.[13-16] Molecular hybridization is beneficial as different targets are activated by a single molecule and is particularly interesting where treatment is limited to few commercial drugs or in cases where discovered

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bioactive compounds present high toxicity or pharmacokinetic and pharmacodynamic limitations.[17,18] The hybrid molecules can be designed in different ways as described follows:

- *Metabolically stable hybrids*, in which the desired molecules are tethered *via* linkers or spacers which may or may not be stable under *in vivo* conditions. These conjugates interact preferably at more than one target with effects mostly additive or synergistic for a particular disease.
- *Cleavable molecular hybrids*, which get metabolized *in vivo* with the release of parent drug molecules and have different targets and mechanism of action.
- Molecular hybrids can also be prepared *via* either fusing the drug molecules without introducing a linker or by merging the individual molecules by taking advantage of commonalities in their structure.

Today, much of the world's population is affected with infectious diseases that remain instrumental for debilitating poverty. According to the latest statics published in 2012, 8.7 million people died worldwide in 2008 due to infectious disease.[19] People who lack food, shelter, security and social protection are more vulnerable to infectious diseases since they often lack the most basic measures of prevention and care. Pathogenic microorganisms that include bacteria, viruses, parasites or fungi are the major cause of infectious disease and can spread directly or indirectly, from person to person. Among infectious disease; malaria, tuberculosis (TB) and HIV/AIDS are high on the global agenda while other less important infections include chagas disease, human african trypanosomiasis, trichomoniasis and leishmaniasis.[20]

Malaria is considered to be one of the most dangerous parasitic diseases because of its high morbidity, mortality as well as its socio-economic impacts on the malaria-endemic region. It remains as a chief cause of illness and death in tropical and subtropical countries including Africa, Asia and South America. Approximately 90 percent cases of malaria are found in sub-Saharan Africa. According to World Health Organization (WHO) report 2012, 3.4 billion people in 103 countries are at a risk of infection with 207 million malaria cases and 627,000 deaths, with the majority of victims being pregnant women or children less than age of five years.[21] The disease is caused by genus *Plasmodium* and among 200 *Plasmodium* species, only five species infect humans *viz. Plasmodium falciparum*, *P. vivax*, *P. ovale, P. malariae* and *P. knowlesi*, with *P. falciparum* being the most virulent. Historically, safe and cheap drug chloroquine (CQ) was extensively used for the treatment of malaria due to its excellent clinical efficacy, restricted host toxicity and simple cost-effective synthesis. CQ is believed to target ferriprotoporphyrin IX (FPIX) heme inside the digestive vacuole (DV) of the parasite. FPIX, being toxic to the parasite in its free state, is sequestered as a non-toxic crystalline hemozoin, also called "malarial pigment." CQ forms a complex with FPIX, resulting in the accumulation of toxic hematin (Fe(III)PPIX) within the digestive vacuole and instigating the death of parasite.[22-29] However, the onset of resistant strain to the available antimalarial drugs including CQ is responsible for the global rise of malaria and offers strong impetus for the development of new antimalarials.[30,31] The development of resistance of *P. falciparum* to CQ is mainly attributed to the mutations in the *P. falciparum* chloroquine resistant transporter gene (PfCRT), a protein involved in the efflux of drug and proton equilibrium across the membrane of the digestive vacuole, resulting in poor accumulation of CQ in acidic food vacuole of the parasite.[32,33] Currently, WHO recommends Artemisinin based combination therapy (ACT) comprising of artemisinin and its semi-synthetic derivatives in combination with the existing drugs for the treatment of malaria. This drug regimen is considered successful against both the CQ-sensitive as well as CQ-resistant strains of malaria.[34-36] However, recent reports of the development of clinical resistance to ACT in the Southeast Asia threatened this combination therapy.[37]

4-Aminoquinoline hybridization is now considered as an attractive and viable strategy for preventing and delaying the emergence of drug resistance along with the improvement in efficacy.[38-42] The success of quinoline-hybridization strategy was exemplified by several potential antimalarials such as trioxaferroquines,[43] trioxaquines,[44] artemisinin-quinine hybrid,[45] 4-aminoquinoline based tetraoxanes,[46] clotrimazole-based-4-aminoquinoline[47] and isatin-4-aminoquinoline hybrids.[48] The present review article encompasses the recent developments on the utility of 4-aminoquinoline-hybridization towards the development of novel antimalarials. A special focus has been given on the Structure-Activity Relationship, IC_{50} values against CQ-sensitive and resistant strains, *in vivo* evaluation data and cytotoxic studies of the promising candidates emerged from this strategy.

2. 4-aminoquinoline-based antimalarial conjugates

2.1 4-Aminoquinoline-Chalcone conjugates:

Chalcones and dienones are structurally linked compounds and revealed to exhibit notable *in vitro* and *in vivo* antimalarial activity[49-52] by acting as inhibitors of either plasmodial aspartate proteases,[53] cysteine proteases[54] or permeability pathways initiated into erythrocyte cell membranes by malaria parasite.[55] Chibale and co-workers has applied molecular hybridization strategy *via* Cu(I)-catalyzed cycloaddition reaction of terminal alkynes and azides for the synthesis of series of 1*H*-1,2,3-triazole linked chalcone and dienone conjugates containing aminoquinoline and nucleoside templates (**Fig. 1**).[56] The synthesized hybrids were screened for their antimalarial activity against the CQ-sensitive (D10) and CQ-resistant (Dd2 and W2) strains of *P. falciparum*. Notably, the azidothymidine (AZT) conjugates with both chalcones and dienones did not show any improvement in the antimalarial activity over their acetylenic precursors while retention of activity was observed in most of the cases. The quinoline-hybridization approach led to the identification of highly potent hybrids, with the most active conjugate **4a** having sub-micromolar IC_{50} values of 0.04, 0.07 and 0.09 μM against tested D10, Dd2 and W2 strains of *P. falciparum*. Cytotoxicity against Chinese Hamster Ovarian cell line was determined and the hybrid **4a** proved to be non-cytotoxic even at the highest concentration tested (100 μM).

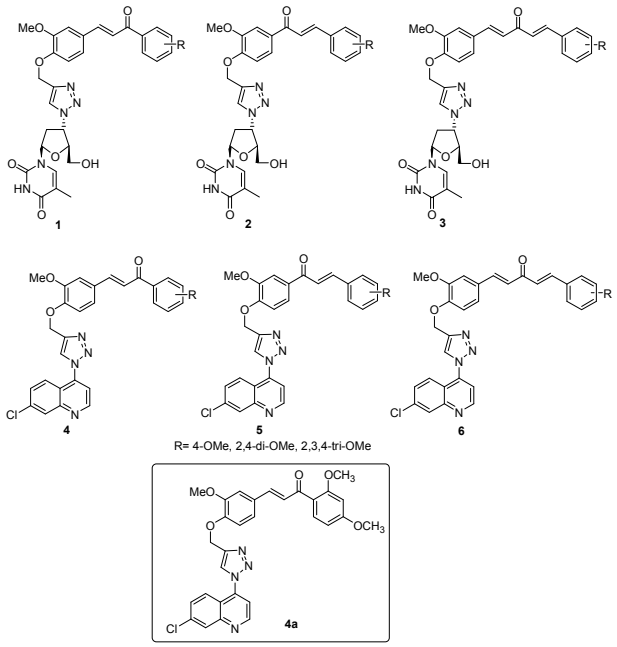
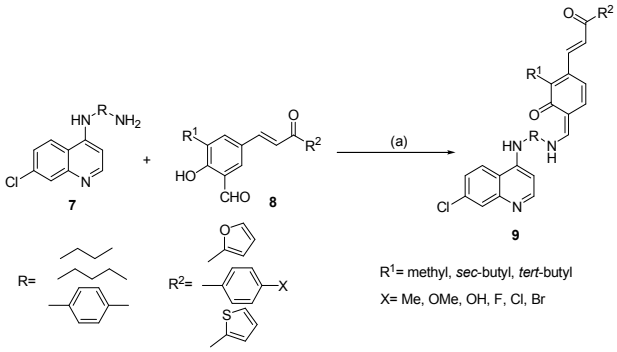


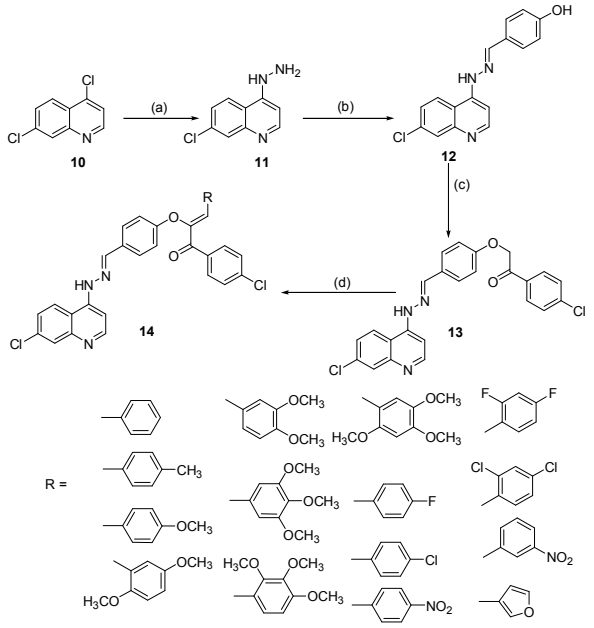
Fig. 1 General structures for triazole-linked chalcone and dienone hybrid compounds comprising AZT and aminoquinoline with the most active hybrid **4a**

Sashidhara and co-workers have utilized an uncommon approach towards the synthesis of keto-enamine tethered chalcone-4-aminoquinoline conjugates **9**.[57] The synthesized conjugates were evaluated for their antimalarial potential against CQ-sensitive (3D7) strain of *P. falciparum* (**Scheme 1**). The promising compounds from *in vitro* assay were further screened for their antimalarial efficacy against *P. yoelii* (CQ-resistant N-67 strain) in Swiss mice. Two of the synthesized conjugates displayed suppression of 99.9 percent parasitemia on day 4. Mechanistically, the test compounds exhibited antimalarial mode of action similar to that of CQ as confirmed by inhibition of β-hematin formation studies.



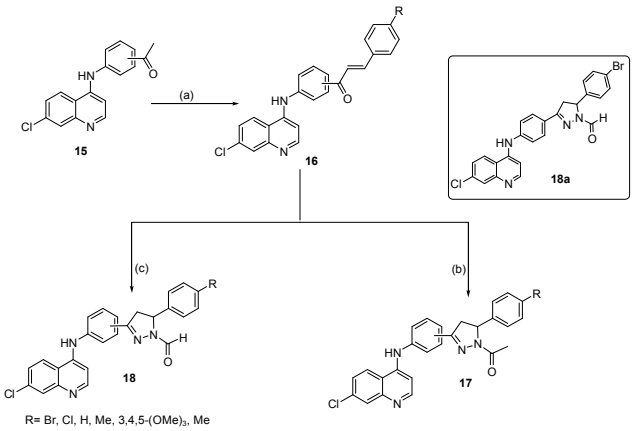
Scheme 1: Reagents and conditions: (a) Ethanol, rt, 10 min.

The above work was further extended towards the synthesis of 4-aminoquinoline-chalcone conjugates by following sequence of synthetic steps as shown in **Scheme 2** along with their antimalarial evaluation.[58] Most of synthesized conjugates showed improved antimalarial profile against CQ-resistant K1 strain when compared to CQ. Structure activity relationship (SAR) studies demonstrated dependence of activity profile on the substitution pattern as well as the number of substituent present on the phenyl ring. The presence of electron releasing groups (R = -CH_3, -OCH_3) have shown to improve the activity profiles while replacing them with the electron withdrawing groups (R = -Cl, -NO_2) reduced the antimalarial activity with the exception of fluorine. β-hematin studies were also carried out for the synthesized conjugates with the three compounds being most active in inhibition of β-hematin formation having IC_{50} values of 3.46, 3.52 and 3.74 μg/ml.



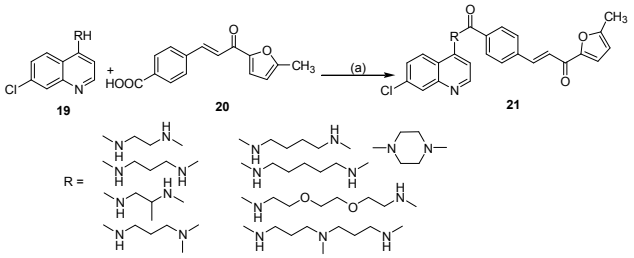
Scheme 2. Reagents and conditions: (a) Hydrazine hydrate, EtOH, reflux, 8 h; (b) 4-hydroxybenzaldehyde, EtOH, reflux, 2-3 h; (c) 4-chlorophenacyl bromide, K_2CO_3, acetonitrile, rt, 2 h; (d) different substituted aldehydes, 10% methanolic KOH, rt

Insuasty *et al.* has utilized 7-chloroquinoline-amino-chalcone hybrids (**16)** for the preparation of a series of *N*-acetyl and *N*-formyl-pyrazoline derivatives (**17** and **18**) in acceptable to good yields *via* cyclo-condensation reaction using hydrazine hydrate under acidic conditions (**Scheme 3**). The synthesized conjugates were bio-evaluated for their antimalarial and anticancer profiles.[59] The anticancer profiles against 60 cell lines revealed that GI_{50} values for most of these conjugates range from 0.13 to 0.99 μM. The antimalarial activity performed against NF54 strain of *P. falciparum* revealed that one of the *N*-formyl-pyrazoline derivative **18a** exhibited percentage inhibition of 50.8%.



Scheme 3. Reagents and conditions: (a) KOH, MeOH, rt; (b) hydrazine hydrate, acetic acid, reflux; (c) hydrazine hydrate, formic acid, DMF, reflux

A series of 4-aminoquinolinyl-chalcone amides **21** were synthesized by N'Da through condensation of carboxylic acid-functionalized chalcone with aminoquinolines (**Scheme 4**) along with their screening against CQ-sensitive (3D7) and CQ-resistant (W2) strains of *P. falciparum*. Cytotoxicity against WI-38 cell line of normal human fetal lung fibroblast was also evaluated.[60] The IC_{50} values of the synthesized conjugates ranging between 0.04-0.5 μM and 0.07-1.8 μM against 3D7 and W2, respectively. SAR studies revealed the increase in antimalarial activity of the amides with the increase in lipophilicity and alkyl chain length. Moderate to high toxicity towards the mammalian cells was observed for these conjugates. The most active compound featuring 1,6-diaminohexane as linker was two-folds potent than CQ against the 3D7 and W2 strains despite its predicted high lipophilicity, low solubility and poor absorption properties.



Scheme 4. Reagents and conditions: (a) CDI, DCM:DMF, rt, 24 h

2.2 4-Aminoquinoline-Pyrimidine conjugates:

Rawat and co-workers reported the preparation of a library of 4-aminoquinoline-pyrimidine conjugates in an attempt to search for more active molecule which shows effectiveness against both CQ-sensitive and CQ-resistant strains of *P. falciparum*.[61] Among synthesized compounds, eleven conjugates displayed enhanced antimalarial activity than CQ against both D6 and W2 strain, while four conjugates (**22**, **23**, **24**, and **25**, **Fig. 2**) showed better activity against both CQ-sensitive as well as CQ-resistant (D6 and W2) strain of *P. falciparum* in comparison to pyrimethamine. Most of the conjugates were found to be non-cytotoxic up to a concentration of 60 μM, while others showed mild toxicities. Most of the conjugates exhibited high selective index with compounds **22** and **25** having IC_{50} values of 0.005 and 0.006 μM against D6 strain and 0.03 and 0.06 μM against W2 strain of *P. falciparum*, were selected for *in vivo* studies, have shown outstanding activity in a mouse model of *P. berghei* without any toxicity.

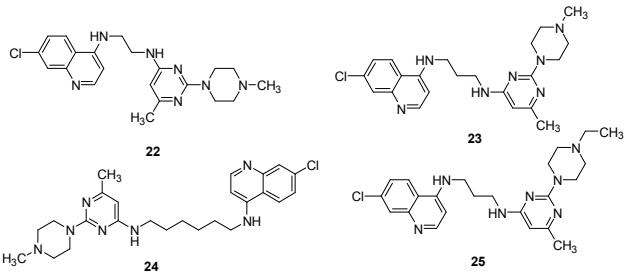
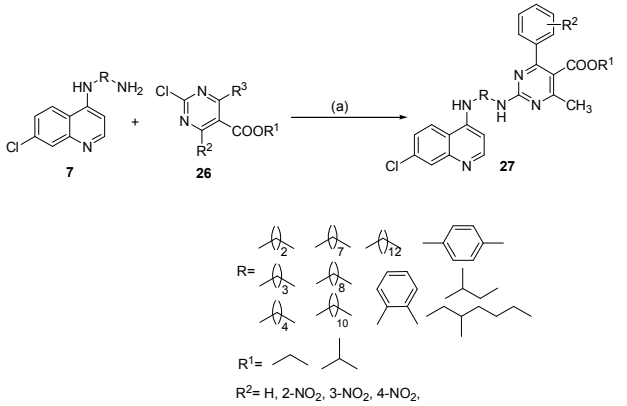


Fig. 2 Most Potent 4-aminoquinoline-pyrimidine conjugates **22**, **23**, **24** and **25**

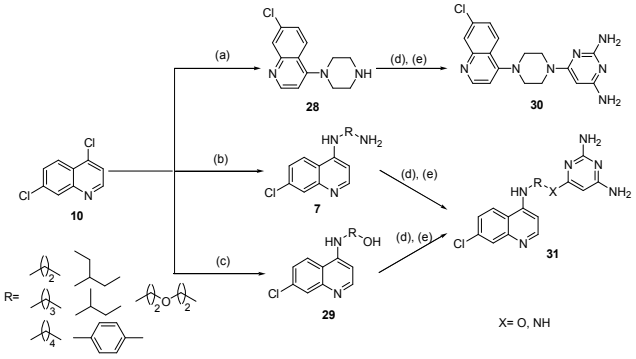
Singh and co-workers in a recent communication, has shown the synthesis of molecular conjugates **27** based on 7-chloro-4-aminoquinoline **7** and 2-aminopyrimidine **26** motifs, as shown in **Scheme 5**.[62] The rationale behind using 2-aminopyrimidine was because of its well established antimalarial potential as evidenced by structurally related drug pyrimethamine. The antiplasmodial profile of the synthesized conjugates were in nanomolar range with the most potent hybrid exhibiting an IC_{50} value of 3.6 nM; 56-fold less compared to CQ against CQ-resistant K1 strain. Almost all of the synthesized compounds were cytotoxic and the binding studies with DNA implying their strong affinity for target parasite type AT rich pUC18 DNA. The active conjugates also showed good inhibitory activity for β-haematin formation, suggestive of the fact that the observed antiplasmodial potential of the conjugates in the present case is because of their ability to act on multiple targets.



Scheme 5. Reagents and conditions: (a) K_2CO_3, THF, 48 h, rt

A range of 7-chloroquinoline-pyrimidine hybrids *viz.* **30** and **31** were synthesized by N'Da and co-workers *via* aromatic nucleophilic substitution reaction of 4-aminoquinolines with 2,6-diamino-4-chloropyrimidine and evaluated their antiplasmodial potential. The conjugates were evaluated alongside CQ, pyrimethamine (PM) and their fixed combinations *viz.* CQ/PM (1:1) and CQ/PM (1:4) against the CQ-susceptible D10 and -resistant Dd2 strain of *P. falciparum*

(**Scheme 6**).[63] The cytotoxic profiles of the synthesized scaffolds were also evaluated against Chinese Hamster Ovarian (CHO) cell line. All the synthesized hybrids showed activity against both D10 and Dd2 strains with good selective index. The most potent compound of the series **30** with piperazine as linker showed comparable activity to that of PM and CQ against D10 strain (IC_{50} = 0.07 μM) while a three folds better potency against CQ against Dd2 strain (IC_{50} = 0.157 μM) has been observed.



Scheme 6. Reagents and conditions: (a) Piperazine, DMF, 80-135 °C, 5 h; (b) diaminoalkane/aryldiamine, neat or DMF, 80-150 °C, 24 h; (c) aminoalcohol, neat, 120 °C, 24 h; (d) NaH, DMF, rt, 1 h; (e) 2,6-diamino-4-chloropyrimidine, DMF, 135 °C, 16-24 h

Further, a library of piperazine tethered 4-aminoquinoline-pyrimidine conjugates were prepared and screened for their antimalarial profile over CQ-sensitive (D6) and CQ-resistant (W2) strains of *P. falciparum* while cytotoxicity was evaluated against mammalian vero cell line.[64] Nine conjugates were shown to have superior antimalarial potency against both the strains with IC_{50} values ranging from 0.13 to 0.14 μM. Out of these, compound **32** (**Fig. 3**) proved to be the most potent among the synthesized conjugates and its antimalarial activity was found to be 2.5 fold more than standard drug CQ. All the synthesized conjugates were non-cytotoxic against Vero cell line.

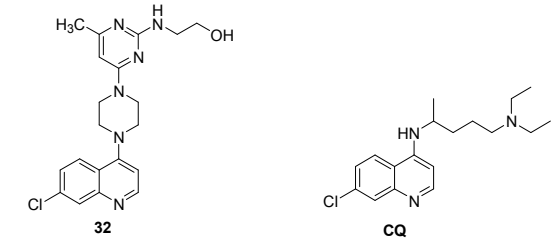
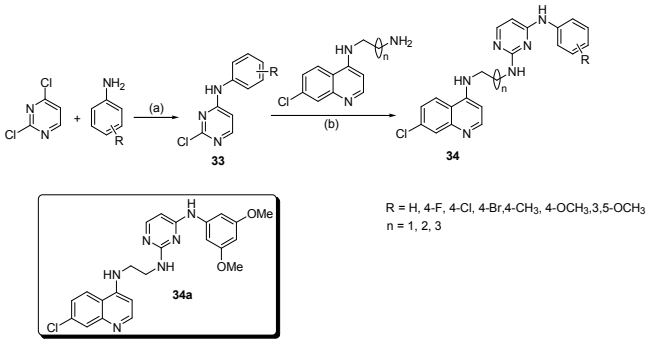


Fig. 3 Most potent Piperazine tethered 4-aminoquinoline-pyrimidine conjugate **32** and **CQ**

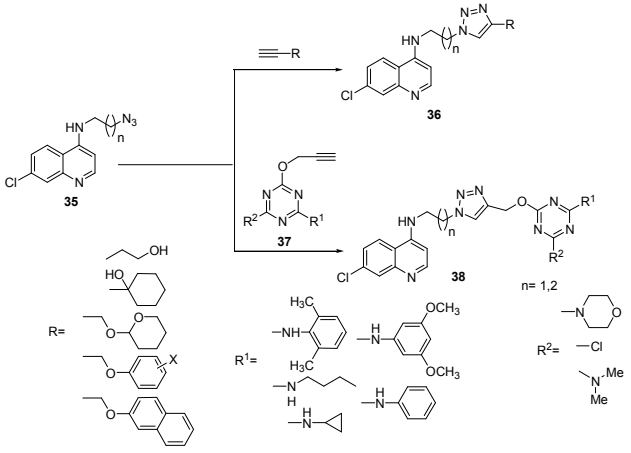
The above work was further extended for the preparation of 4-aminoquinoline-pyrimidine hybrids **34** as shown in **Scheme 7**. All the synthesized compounds were screened for their antiplasmodial activity against D6 and W2 strains of *P. falciparum* depicting activity in nano-molar range.[65] Numerous compounds were found to be nontoxic to the mammalian cell lines and showed promising *in vitro* antimalarial activity over both CQ-sensitive and -resistant strains with high values of selective index. Most potent conjugate **34a** with an IC_{50} value of 0.033 and 0.058 μM against D6 and W2 strains of *P. falciparum*, was chosen for *in vivo* studies and exhibited significant suppression of parasitemia. The heme binding studies confirmed heme as one of the possible targets of these hybrids with heme forming a stable 1:1 complex with these conjugates. Potent compounds from *in vitro* antimalarial activity data were selected for molecular docking simulations and showed good interaction with the binding sites of PfDHFR.



Scheme 7. Reagents and conditions: (a) TEA, EtOH, rt, overnight; (b) K_2CO_3, NMP, 140-150 °C, 10-12 h

2.3 4-Aminoquinoline-Triazine conjugates:

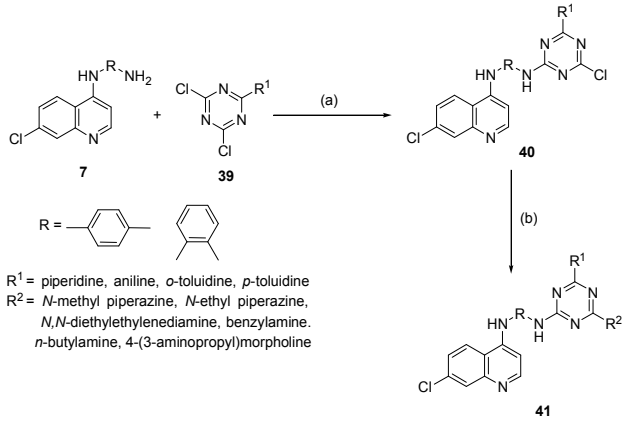
Rawat *et al.* described the synthesis of a library of 4-aminoquinoline-1,2,3-triazoles **36** and 4-aminoquinoline-1,2,3-triazole-1,3,5-triazine conjugates **38** by utilizing Huisgen-1,3-dipolar cycloaddition reaction of 4-azido-7-chloroquinoline with variedly substituted terminal alkynes (**Scheme 8**).[66] It has been hypothesized that amalgamation of a basic nucleus *viz*. triazine with 4-aminoquinoline would enhance the accumulation of conjugate in the digestive food vacuole targeting *P. falciparum* dihydrofolate reductase (PfDHFR). However, the antimalarial profile of the synthesized conjugates against CQ-sensitive D6 and CQ-resistant W2 strain revealed that none of the hybrids exhibit better activity compared to CQ.



Scheme 8. Reagents and conditions: (a) $CuSO_4.5H_2O$, Sodium ascorbate, *t*-BuOH:H_2O (1:1), 40 °C, 3 h

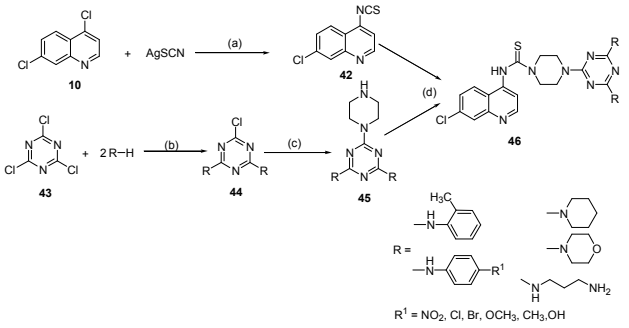
The above study was further extended by Chauhan and co-workers in the synthesis of 4-anilinoquinoline-triazine derivatives (**40** and **41**) using readily available and cheap starting materials

(**Scheme 9**). The compounds were assessed for their antiplasmodial efficacy against CQ-sensitive 3D7 strain of *P. falciparum* as well as for their cytotoxicity towards mammalian vero cell line.[67] The most promising and non-cytotoxic compounds from the preliminary studies were chosen for *in vivo* screening in Swiss mice infected with CQ-resistant N-64 strain of *P. yoelli*. These studies resulted in the identification of two orally active compounds having piperidine functionality. The results obtained are suggestive of the fact that the antimalarial activity in these conjugates can be attributed to the metabolic stability of piperidine functionality. The selected conjugates were further investigated for their ability to inhibit β-hematin formation. All the tested hybrids exhibited better inhibitory activities of β-haematin formation (IC_{50} ranging from 2.65 to 3.16 μM) than CQ (IC_{50} = 3.65 μM) which revealed that apart from targeting heme, there might be the possibility of other mechanism of action.



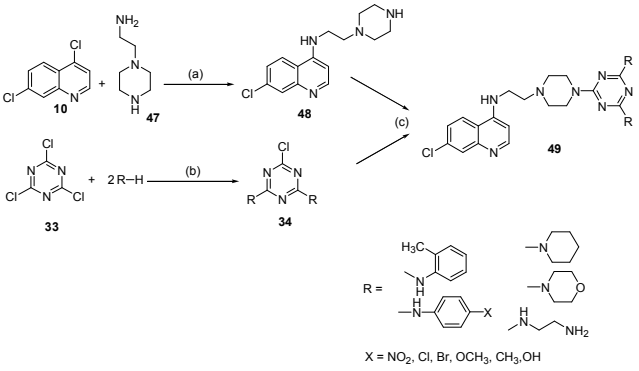
Scheme 9. Reagents and conditions: (a) Mono-substituted triazines, THF, reflux, 8 h; (b) various amines, THF, reflux, 5 h

The use of triazine functionality for the synthesis of molecular conjugates with antimalarial potential was further elaborated by Bhat and co-workers in the synthesis of variedly functionalized 1,2,3-triazine-4-aminoquinoline conjugates **46** attached *via* thiourea functionality as the linker (**Scheme 10**). The determination of their antimalarial efficacy was done against CQ-sensitive (3D7) and CQ-resistant (RKL-2) strain of *P. falciparum*.[68] The observed SAR revealed a preference for electron withdrawing substituents *viz.* bromo- and hydroxyl groups while the presence of electron donating substituents including methyl and methoxy adversely affected the activity profiles. The observed activities were further substantiated by docking simulations performed by using ligand fit module within Discovery Studio 2.5 on wild and quadruple mutant strain of *P. falciparum* dihydrofolate reductase thymidylate synthase (pf-DHFR-TS).



Scheme 10. Reagents and conditions: (a) Reflux with stirring for 18 h at 90-120 °C; (b) dry acetone, 0-5 °C, 1 h, then 40-45 °C for a further 3 h; (c) piperazine, 1,4-dioxane, 120-130 °C, 5-6 h; (d) dry acetone, 40-45 °C, 18 h

Further extension of the above work resulted in the synthesis of 4-aminoquinoline-1,3,5-triazine derivatives **49** connected through non-ionizable covalent linkers (**Scheme 11**).[69] The antiplasmodial profile again showed a similar SAR as observed earlier with a marked preference for electron withdrawing substituents for good activity. Further, addition of basic moieties such as morpholine, piperidine and 1,3-diaminopropane in the synthesized conjugates has shown to enhance the antimalarial activity. The evaluation results were correlated using docking studies carried out on wild and quadruple mutant pf-DHFR-TS. In wild type strain, most of the hybrids revealed hydrophobic interaction between the phenyl ring of conjugates with the Phe58 residue as well as σ-π interaction between the 1,3,5-triazine ring and Leu46 residue. Similarly, a hydrophobic interaction was observed between the phenyl ring and Phe58 in addition to H-bond formation between 1,3,5-triazine ring and Ser111 in the quadruple mutant of pf-DHFR-TS. Further, it was observed that the quinoline moiety was engaged in the formation of +–π interaction with Arg59.

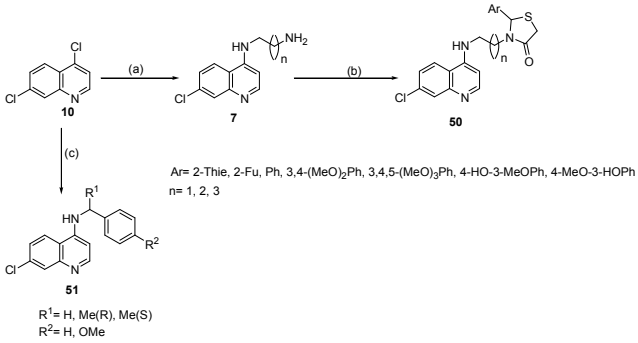


Scheme 11. Reagents and conditions: (a) Reflux 1 h at 80 °C followed by 6-8 h at 120-130 °C; (b) 1,4-dioxane 0-5 °C, 1 h, 40-45 °C, 3 h; (c) 1,4-dioxane, 120-130 °C, 6-7 h

2.4 4-Aminoquinoline-Thiazolidine conjugates:

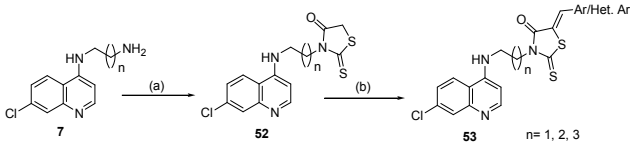
An alternative pathway for obtaining new and promising antimalarial compounds was disclosed by Kouznetsov and co-workers. A library of 21 heterocyclic scaffolds consisting of either *N*-(aminoalkyl)thiazolidin-4-one-4-aminoquinoline conjugates **50** or substituted *N*-benzylamino-7-chloroquinolines **51** were prepared by following sequence of steps as depicted in **Scheme 12**.[70] The antimalarial evaluation against 3D7 and Dd2 strains have shown

that four of the *N*-benzylamino-7-chloroquinoline derivatives **51** are up to 3-folds more active compared to the standard drug, CQ. Non-specific cytotoxicity assay on J774 murine macrophages and HepG2 cells (human heptocellular carcinoma cell line) proved their high selectivity index and hence the potential for *in vivo* evaluation.



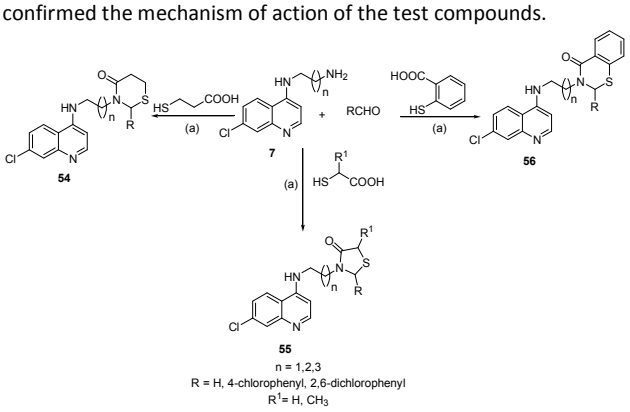
Scheme 12. Reagents and conditions: (a) diaminoalkane, 80 °C for 1 h, 140-150 °C for 6-7 h; (b) ArCHO, $HSCH_2COOH$, PhMe, 4-5 h reflux; (c) substituted *N*-benzylamine, K_2CO_3, DMF, 140 °C, 10 h

Chauhan and co-workers has recently explored the synthesis of 4-aminoquinoline-rhodanine hybrids along with their *in vitro* antimalarial efficacy against K1 and 3D7 strains of *P. falciparum*, as well as their cytotoxicity against vero cell line (**Scheme 13**).[71] Although none of the hybrids was more potent than CQ against 3D7 strain, four of the tested conjugates showed antimalarial activity comparable to CQ with IC_{50} values in the range of 13.2-45.5 nM against K1 strain along with high selectivity. Furthermore, some of the hybrids were also tested for their anti-mycobacterial potential against $H_{37}Rv$ strain of *M. tuberculosis* with three of the potent compounds exhibiting minimum inhibitory concentration (MIC) value of 6.25 μM. In addition, inhibition of *β*-hematin formation studies disclosed that the test conjugates were more active than CQ in the inhibition of hemozoin formation clearly validating their mechanism of action.



Scheme 13. Reagents and conditions: (a) CS_2, $BrCH_2COOC_2H_5$, acetonitrile, rt, 3-5 h, (b) acetic acid, ammonium acetate, aromatic/ hetroaromatic aldehydes, 90 °C, 4-6 h

A series of differently substituted 4-aminoquinolines having thiazolidin-4-ones (**54**), [1,3]thiazinan-4-ones (**55**) or 2,3-dihydrobenzo[e][1,3]thiazin-4-ones (**56**) at the terminal amino functionality was synthesized *via* three component reaction between 4-aminoquinolines, aryl aldehydes and appropriate mercapto acids (**Scheme 14**).[72] Among the compounds tested against NF-54 strain of *P. falciparum*, nine showed IC_{50} values ranging between 0.013-0.98 μM. The promising compounds from *in vitro* assay were further selected for *in vivo* activity in Swiss mice using N-67 strain of *P. yoelli*. The most potent compound from *in vivo* analysis suppressed 81 percent parasitemia on the day 4 compared to 100 percent suppression exhibited by CQ. SAR studies showed that the lateral side chain modification of 4-aminoquinoline in these conjugates was well tolerated with the best results obtained with two or three carbon chain as linker. The biochemical studies confirmed their association with hematin and hence confirmed the mechanism of action of the test compounds.



Scheme 14. Reagents and conditions: (a) DCC, THF, rt or PhMe, reflux

2.5 4-Aminoquinoline-Clotrimazole conjugates:

With the aim to develop new antimalarials, Gemma and co-workers in a recent communication have identified a new polyaromatic antimalarial pharmacophore based on clotrimazole scaffold.[73] Clotrimazole is a popular antimycotic drug with weak antimalarial activity (IC_{50} = 0.55 μM) against W2 strain of *P. falciparum*. However, its peculiar structural features *viz*. (i) the presence of an imidazole nucleus, identified to mediate electron transfer reaction and (ii) the triphenylmethyl system known to stabilize radical intermediate, makes it as an ideal candidate to interact with haemoglobin-derived ferro-protoporphyrin (Fe(II)-FP) complex in the food vacuole of parasite. Consequently, a number of polyaromatic pharmacophores structurally related to clotrimazole have been synthesized with an extension towards clotrimazole-4-aminoquinoline conjugates. The antimalarial evaluation of the synthesized scaffolds showed dependence upon some key structural features *viz*. (i) the protonatable side chain, (ii) the imidazole ring, and (iii) the aryl/heteroaryl system. The most promising compounds **57**, **58** and **59** (**Fig. 4**) were further evaluated to access their *in vivo* efficacy against *P. chabacidi* in CDI mice after oral administration in the 4 day suppression test. The results showed that the compounds **57** and **58** have low propensity to produce rapid resistance along with *in vivo* activity against plasmodia and oral bioavailability.

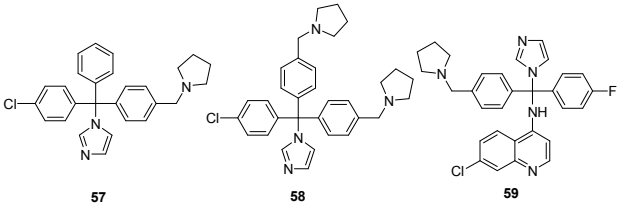


Fig. 4 Most potent compounds **57**, **58** and **59**

The above protocol was further extended towards the synthesis of a series of antimalarial hybrids by combining the 4-aminoquinoline unit with that of clotrimazole as depicted in **Fig. 5**.[74] The antimalarial studies were carried out *in vitro* against CQ-sensitive and -resistant strains as well as *in vivo* in a rodent malaria model. The compound **63** and **64** displayed strong antimalarial activity against *P. berghei* after oral administration. The compounds have shown to interfere with the process of heme detoxification as confirmed by BHIA assay. Cytotoxicity, genotoxic potential and effects on cardiac function of lead compounds were also assessed with the compound **63** emerged as a good hit because to its promising antimalarial potency and an optimal half-life in mice.

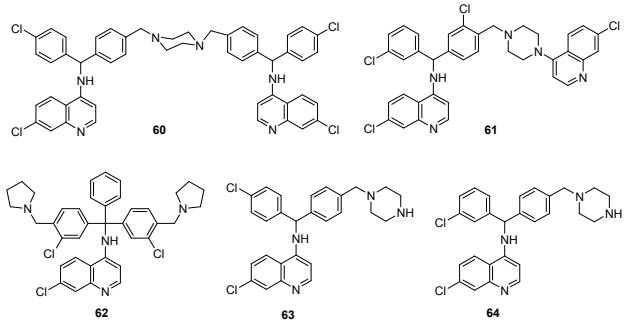


Fig. 5 Molecular conjugates **60**, **61** and **62** of 4-aminoquinoline and clotrimazole along with their potent precursors **63** and **64**

2.6 4-Aminoquinoline-Azithromycin conjugates:

Azithromycin, a semi-synthetic macrolide antibiotic, was recently discovered as a slow acting antimalarial[75,76] that wields its activity *via* inhibiting protein synthesis on prokaryote-like ribosome in *P. organelle*.[77] Currently, it is in clinical trials and has been used as a combination partner with different antimalarials. Peric and collaborators has employed molecular hybridization strategy for the synthesis of azithromycin-quinoline antimalarial conjugates.[78] Forty two new azithromycin analogues and azithromycin-quinoline conjugates having amide and amine functionalities were synthesized using simple and economical chemical procedures. These scaffolds displayed 100-folds improvement in *in vitro* potency with high selectivity, pharmacokinetics (PK) and *in vivo* efficacy over azithromycin against *P. falciparum* TM91C235 strain, a multidrug resistant clone from Southeast Asia. The screening of the promising compounds in a mouse efficacy model resulted in the identification of five scaffolds *viz.* three amine (**65**, **66**, **67**) and two amide (**68**, **69**) derivatives exhibiting better *in vivo* efficacy compared to azithromycin (**Fig. 6**). The most potent of the tested compounds **65** (**Fig. 6**) showed 100 folds better *in vitro* activity with excellent pharmacokinetic parameters curing mice more efficiently than azithromycin.

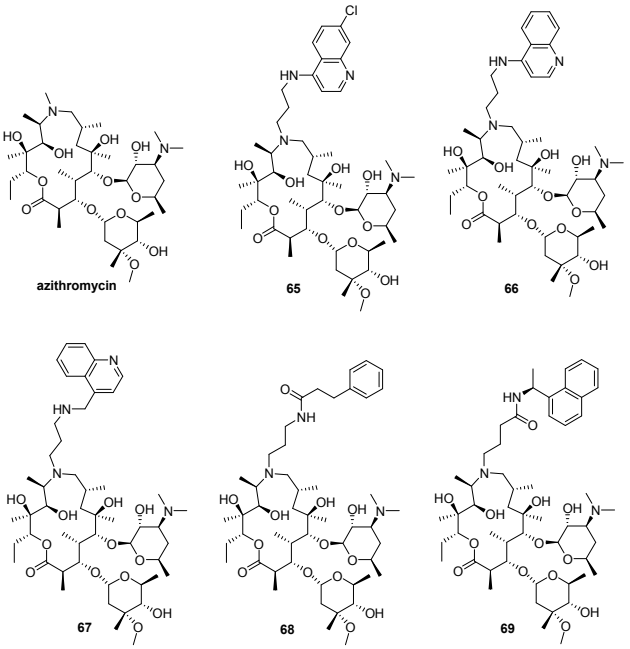


Fig. 6 Structure of azithromycin and potent azalide hybrids used for *in vivo* study in the mouse model

Peric *et al.* further extended this concept towards the development of azalide class of antimalarials *via* synthesis of 2'-*o*-substituted-9-deoxo-9a-methyl-9a-aza-9a-homoerythromycin A derivatives covalently linked to varied substituted quinolines at position 2' as depicted in **Fig. 7**.[79] Antimalarial profiles of the test conjugates against CQ-sensitive 3D7 strain showed 100-fold improvement over azithromycin while 48-fold enhancement was observed against CQ-resistant W2 strain of *P. falciparum*. These results have facilitated these macrolides for the preclinical development of malaria-specific agents.

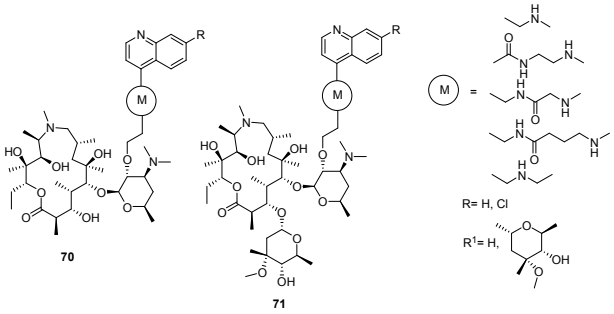


Fig. 7 General structure of hybrids of 2'-O-substituted-9-deoxo-9a-methyl-9a-aza-9ahomoerythromycin A and quinoline

2.7 4-Aminoquinoline-β-lactam conjugates:

A series of 1*H*-1,2,3-triazole linked 7-chloroquinoline-β-lactam conjugates **72** and **73** were synthesized by Kumar and co-workers utilizing click chemistry as shown in **Fig. 8**. Antimalarial potency of the synthesized conjugates against W2-resistant strain of *P. falciparum* was found to be dependent on the substituent present at *N*-1 position of the β-lactam ring and the presence of bis-triazole at C-3 position. The observed antimalarial efficacy was further

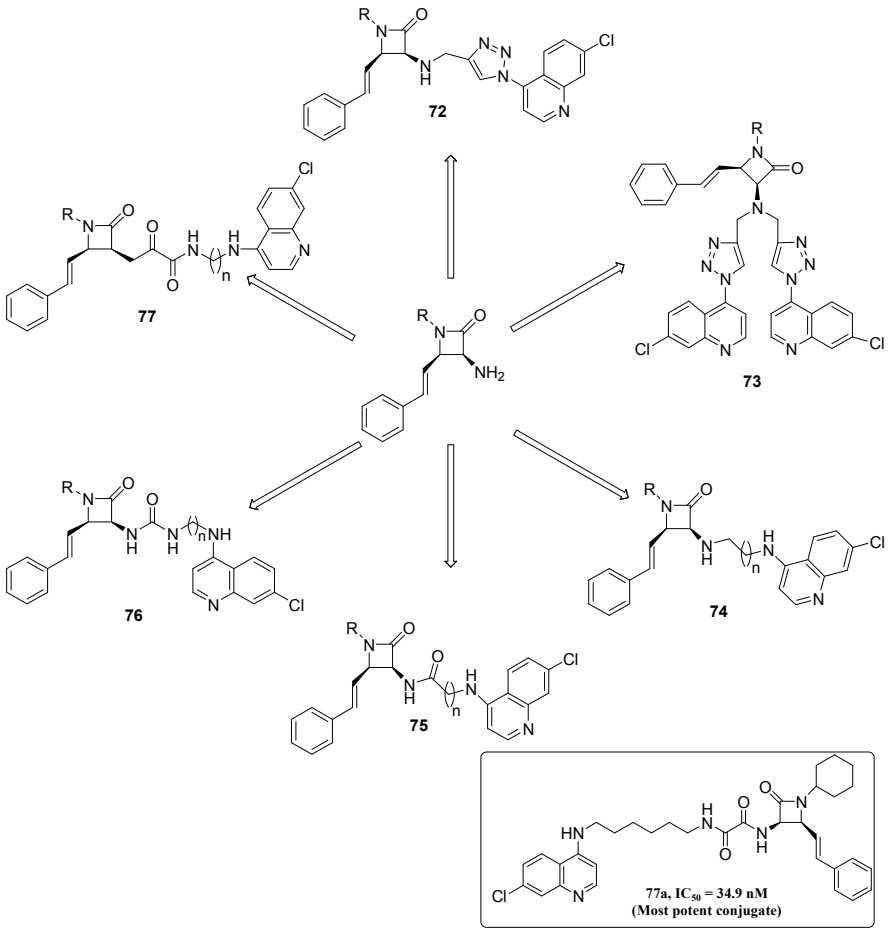
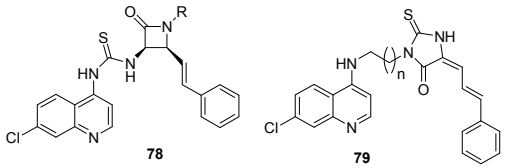


Fig. 8 β-lactam-4-amino-quinoline conjugates

validated by docking studies through inhibition of *P. falciparum* dihydrofolate reductase (PfDHFR).[80]

On the basis of these preliminary results, Kumar *et al.* further extended their work on 4-aminoquinoline hybridization by synthesizing 4-aminoquinoline-β-lactam conjugates linked *via* a diverse range of linkers *viz.* amide[81] (**75**, **Fig. 8**) and non-ionizable covalent bonds (**74**, **Fig. 8**),[81] urea[82] (**76**, **Fig. 8**) and oxalamide[82] (**77**, **Fig. 8**) functionalities along with well modulated alkyl chain lengths. All the synthesized conjugates were screened for their antimalarial potential against W2 resistant strain of *P. falciparum*. Antiplasmodial data revealed that the activity depends on the nature of linker, alkyl chain length and substituent present at *N*-1 position of β-lactam ring. On comparing the potencies among amide and alkyl chain tethered series, the conjugates linked *via* non-ionizable covalent alkyl chain linker proved to be better in inhibiting the growth of *P. falciparum* while the introduction of amide functionality adversely affected the activity profiles. The introduction of urea/oxalamide functionalities, on the other hand, resulted in the improvement in antiplasmodial profiles. The most potent and non-cytotoxic conjugate **77a** (**Fig. 8**), among 7-chloroquinoline-β-lactam series, with best combination of *N*-cyclohexyl substituent at *N*-1, alkyl chain length (n = 6) and oxalamide functionality exhibited an IC_{50} of 39.9 nM.

Further, Kumar *et al.* synthesized a library of C-3 thiourea tethered β-lactam-7-chloroquinoline conjugates (**78**) along with the unexpectedly formed 7-chloroquinoline-thiohydantoin derivatives (**79**) with the endeavour of searching antimalarial structure-activity relationships (**Fig. 9**). The synthesized thiourea-tethered 7-chlorquinoline-β-lactam conjugates **78** were found to be ineffective in inhibiting the growth of *P. falciparum*. However, the unexpected formed product *viz.* 7-chlorquinoline-thiohydantoins **79** demonstrated nanomolar antimalarial activities against W2 strain of cultured *P. falciparum*, with the most potent and non-cytotoxic compound exhibiting an IC_{50} of 39.8 nM. β-hematin formation studies strongly supported the mechanism of their action *via* the inhibition of β-hematin formation having IC_{50} values comparable to those of CQ.[83]

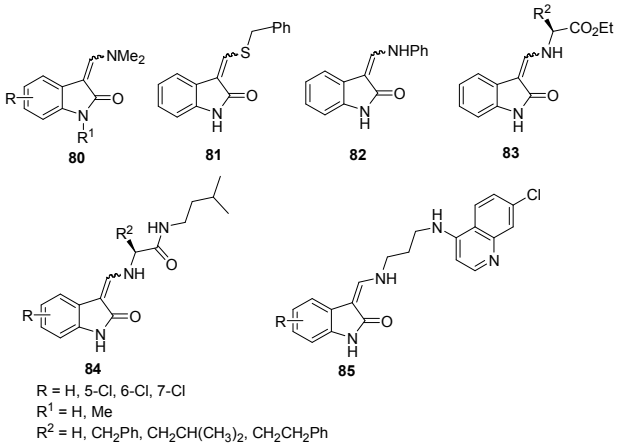


n = 1, 2, 3, 5
R = *p*-C_6H_4-CH_3, C_6H_{11}, *p*-C_6H_4-Cl, *p*-C_6H_4-F, *p*-C_6H_4-Br, C_6H_5

Fig. 9 General structures of *β*-lactam-7-chloroquinoline conjugates, **78** and 7-chloroquinoline-thiohydantoin derivatives, **79**

2.8 4-Aminoquinoline-isatin conjugates:

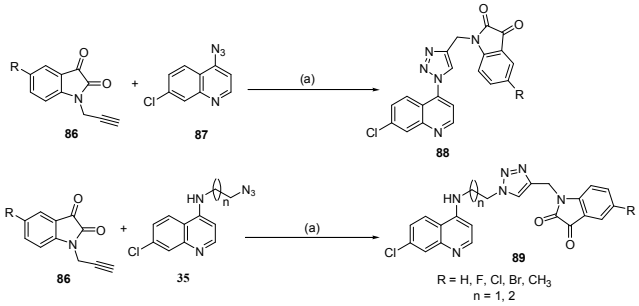
Santos and his colleagues designed, synthesized and evaluated 3-methylene-substituted indolinones (**Fig. 10**, **80**-**85**) as falcipain inhibitors and antiplasmodial agents.[84] Various indolinones having Leu-*i*-amyl recognition moiety were shown to display fair inhibitory activity of *P. falciparum* cysteine protease falcipain-2 and antimalarial potency against W2 strain of *P. falciparum* in low μM range. Importantly, compounds lacking recognition moiety were devoid of FP-2 inhibition and interaction of Leu-*i*-amyl sequence with the S2 pocket. Further, the introduction of a 4-aminoquinoline to the C-3 position of the indolinone-2-one scaffold (**85**) led to a considerable enhancement in the antiplasmodial activity, suggestive of the fact that the 3-methylene-indolinone-2-ones can become forthcoming lead compounds for the development of new antimalarials.



R = H, 5-Cl, 6-Cl, 7-Cl
R^1 = H, Me
R^2 = H, CH_2Ph, $CH_2CH(CH_3)_2$, CH_2CH_2Ph

Fig. 10 General structures of synthesized 3-methylene-substituted indolinone analogues **80**-**85**

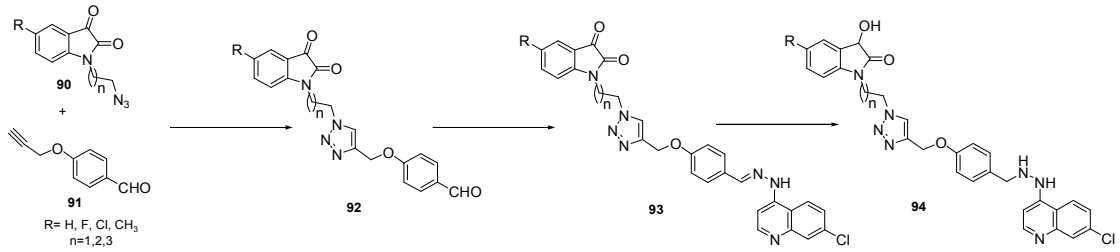
Kumar *et al.* has synthesized a library of isatin-7-chloroquinoline conjugates linked by 1*H*-1,2,3-triazole moiety along with assessment of their antiplasmodial profiles (**Scheme 15**). The tested compounds **88** were devoid of antiplasmodial activity, while **89**, having an alkyl chain between isatin and 7-chloroquinoline moieties, displayed enhancement in antiplasmodial potency. Activity was found to depend on the C-5 substituent of the isatin ring and on the alkyl chain length between isatin and 7-chloroquinoline moieties. The most potent conjugate**,** with an best combination of propyl linker (n=3) and chloro-substitutent at the C-5 position of isatin ring, exhibited an IC_{50} value of 1.21μM.[85]



R = H, F, Cl, Br, CH_3
n = 1, 2

Scheme 15: Reagents and conditions: (a) $CuSO_4$, Sodium Ascorbate, EtOH:H_2O, rt 7 h.

The above work was further extended towards the synthesis of triazole linked isatin-7-chloroquinoline (**93**) and 3-hydroxy-indole-7-chloroquinoline hybrids (**94**) by following synthetic protocol as shown in **Fig. 11** along with their antiplasmodial evaluation against CQ-resistant W2 strain.[86] studies revealed the dependence of activities on the length of alkyl chain but independent on the nature of substituent present at the C-5 position of isatin or indole ring. Most of the conjugates were less active than the standard drug CQ; however the most conjugate with an optimum combination of 3-hydroxy-indole ring and *n*-butyl linker exhibited an IC_{50} value of 69 nM, comparable to that of CQ. Further, Kumar and co-workers synthesized piperazine tethered 7-chloroquinoline-isatin conjugates (**95** and **96**, **Fig. 12**) by both direct nucleophilic substitution and Cu(I)Cl mediated Mannich reaction.[87] All the synthesized conjugates were assessed for their anti-plasmoial, anti-tubercular and cytotoxic evaluation. Analysis of anti-plasmodial activity data against W2 strain showed that none of conjugates were as active as CQ, although some conjugates proved to have good anti-malarial efficacy with IC_{50s} ranging from 0.22-0.27 μM.



R= H, F, Cl, CH_3
n=1,2,3

Fig. 11 General structures of target hybrid compounds

Anti-tubercular evaluation against *M. tuberculosis* revealed dependence of activity profile on the presence of substituent at C-5 position of the isatin ring whereas longer alkyl chain lengths badly affected the efficacy of compounds. Compound **95**, with fluoro substituent was shown to have good anti-TB efficacy with six times more potency than the standard drug, Cephalexin. On the other

hand, Mannich adducts **96**, were found to be ineffective in inhibiting the growth of *M. tuberculosis*. The most potent compound **95a** (**Fig. 12**) with an IC_{50} of 0.22 μM against CQ-resistant strain of *P. falciparum* and 31.62 μM against 3T6 cell line, exhibited a high selectivity index.

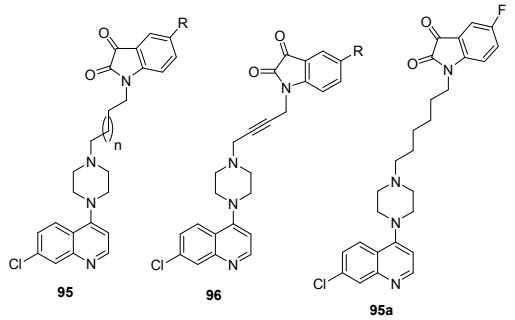


Fig. 12 General structures of piperazine tethered 7-chloroquinoline-isatin conjugates **95** and **96** along with most potent conjugate **95a**

Recently, Kumar *et al.* have synthesized β-amino alcohol tethered isatin-4-aminoquinoline conjugates with the endeavour of searching their antimalarial structure activity relationship. SAR studies have shown a clear preference for shorter alkyl chain length (n=2,3) and the presence of Cl-substituent at the C-5 position of the isatin ring for good activity. Two most potent and non-cytotoxic conjugates 97 and 98 (**Fig. 13**) displayed antiplasmodial potency comparable to that of CQ, with IC_{50} values of 11.7and 13.5 nM, against W2 resistant strain of *P. falciparum*.[88]

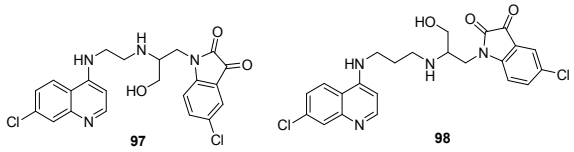


Fig. 13 Two most potent β-amino alcohol tethered isatin-4-aminoquinoline conjugates **97** and **98**

2.9 Organometallic based 4-aminoquinoline conjugates:

Over the past few year, bio-organometallic chemistry have emerged as a quickly growing and maturing part that associates organometallic chemistry to medicine, biology and molecular biotechnology.[89] The replacement of organic ligands with the metal complexes have capability to enhance the activity of biological compounds along with potential advantages such as the synthesis of stable metal complexes having predictable structures, capability to tune ligand affinities in accordance with their electron transfer properties, substitution rates and reduction potentials along with effective biological targeting.[90] Among the implausible number and variety of roles that metals take part in current medicine; cancer[91] and malaria[92] treatments are appreciated as possibly the most important application of metal-based drugs.

In malaria chemotherapy, resistance to established drugs may be overcome by bio-organometallics through new metal specific modes of action. Ferroquine (**99**, FQ, SSR97193, **Fig. 14**), the ferrocene analogue of CQ, is the most pertinent example of a bio-organometallic compound that showed higher antimalarial potency *in vivo* on mice infected with *P. yoelii* NS and *P. berghei* N. It is twenty two times more active against schizontocides than CQ against CQ-resistant strain of *P. falciparum*. The effectiveness of FQ against CQ-resistant strain could possibly be due to its capability to penetrate in the infected cells.[93]

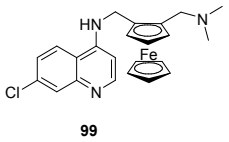
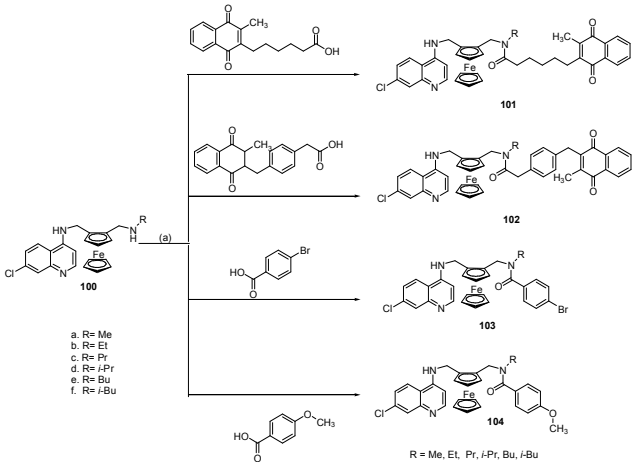


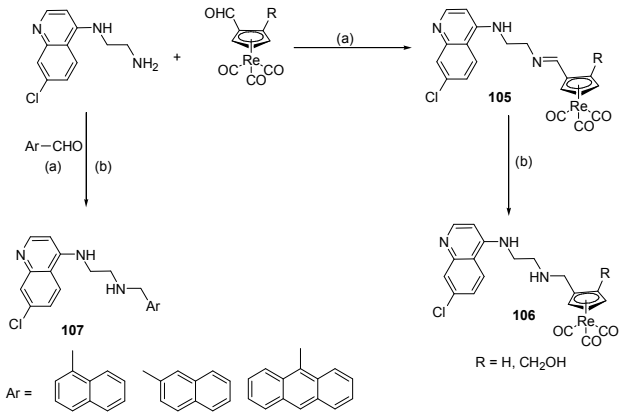
Fig. 14 Structure of Ferroquine (FQ), **99**

Biot *et al.* further utilized the ferroquine pharmacophore as a template for the design of two novel series of ferrocenic antimalarial conjugates comprising of a FQ derivatives hybridized with a glutathione reductase inhibitor (or glutathione depletor) *via* an appropriate cleavable amide bond (**Scheme 16**). *In vitro* antimalarial studies on CQ-susceptible (NF54) and CQ-resistant (K1) strains were conducted, with emphasis on the study of their mechanism of action.[94] Among the series of synthesized conjugates, the compounds **101a**-**101f** were established to exhibit the most potent *in vitro* activity with IC_{50} values in the range of nanomolar. However, their antiplasmodial activity was slightly less than the precursor FQs **100a-100f** possibly due to the cleavage of amide bond and the oxidative metabolism of the side chain of the FQ in the digestive vacuole of the parasite. The observed antiplasmodial activity of FQ was mainly attributed to the high potential of ferrocene core exhibiting a redox mechanism different from the mechanism of action of chloroquine.



Scheme 16. Reagents and conditions: (a) EDCI, DCM, rt

The discovery of ferroquine **99** (FQ, SSR97193) as a promising antimalarial prompted the synthesis of corresponding rhenium organometallics so as to measure the significance of the ferrocenyl group (**Scheme 17**).[95] All the synthesized compounds (organic along with their organometallic counterparts) were assessed for their *in vitro* antiplasmodial potency against the CQ-resistant (W2) and the CQ-susceptible (3D7) strains of *P. falciparum*. The cyrhetrene conjugates (**105** and **106**), however, were found to be less active than the corresponding ferrocene and organic analogues.



Scheme 17. Reagents and conditions: (a) MeOH, rt; (b) $NaBH_4$, MeOH, rt

Orviz *et al.* explored the synthesis of CQ-bridged ferrocenophane analogues of ferroquine in which the terminal nitrogen of the CQ derivative bridged the two cyclopentadienyl rings of ferrocene (**110a**-**110e**) alongside their mono-substituted ferrocene analogues (**109a**-**109e**) and organic fragments (**108a**-**108e**) (**Fig. 15**).[96] All the synthesized compounds were screened for their antiplasmodial potency and were found to be active over both CQ-sensitive and -resistant strains. Mono-substituted ferrocenyl analogues were found to be more active than the CQ-bridged ferrocenyl derivatives while the later compounds *viz.* **110a**-**110e** retained their activity against the CQ-resistant strains. SAR studies revealed the loss of activity with increase in branching while the length of alkyl spacer did not seem to influence their antiplasmodial profiles. The physical properties *viz*. the presence or absence of hydrogen bonding interactions, the degree of rigidity and the lipophilicity of these conjugates were correlated to their biological activity. A balance between lipophilicity and hydrophilicity seemed to influence the biological profile of the conjugates while the structural characteristics such as conformations and size were found essential to overcome the drug resistance.

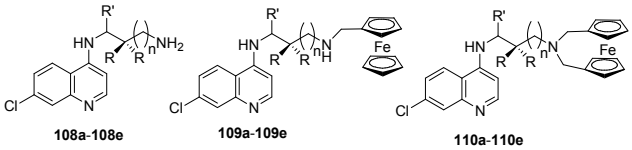


Fig. 15 CQ derivatives **108a**-**108e**, mono-substituted CQ-Ferrocenyl compounds **109a**-**109e**, and 1,1′-disubstituted bridged CQ-Ferrocenyl hybrids **110a**-**110e**

The methodology was further extended with the synthesis of a new class of organometallic antimalarials comprising of a ferrocene nucleus tethered to a CQ analogue and a 1,2,3,5-(diisopropylidene)-α-D-glucofuranose moiety in a 1,1′-heteroannular substitution pattern. Synthesis of these compounds was facilitated by orthogonal functionalization of ferrocene resulting in 1-acetoxy-1′-(1,3-dioxan-2-yl)ferrocene as a key intermediate for the modular introduction of the carbohydrate. This was followed by succeeding reductive amination with CQ derivatives, yielding 1-[3-(7-chloroquinolin-4-ylamino)alkylamino]-1′-[6-(1,2;3,5-diisopropylidene)-α-Dglucofuranosidyl]ferrocenes **117**-**119** (**Fig. 16**). These trifunctional hybrids were assessed for their antiplasmodial activity against CQ-susceptible (D10) and CQ-resistant (Dd2 and K1) strains of *P. falciparum*.[97] No correlation could be established between either the strength of intramolecular H-bonding or the polar surface area and antimalarial potential of these compounds. Antiplasmodial studies indicated that the activity of the synthesized compounds (**117**-**119**) have shown improvement in comparison to their either 1,2-disubstituted regio-isomers **114**-**116** or to monosubstituted ferrochloroquines **111**-**113**. The side chain length linking CQ with the ferrocene molecule did not have any effect on the observed activity.

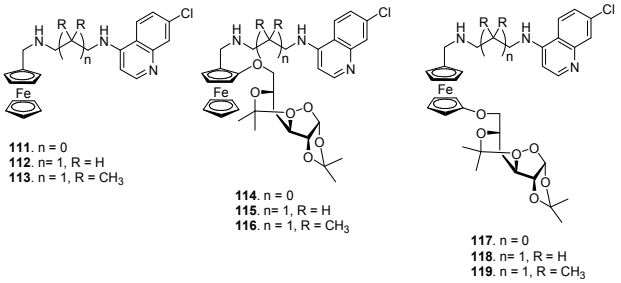


Fig. 16 Mono-, 1,2-disubstituted, 1,1′-disubstituted ferrocenyl chloroquine glucofuranose conjugates **111**-**113**, **114**-**116** and **117**-**119**

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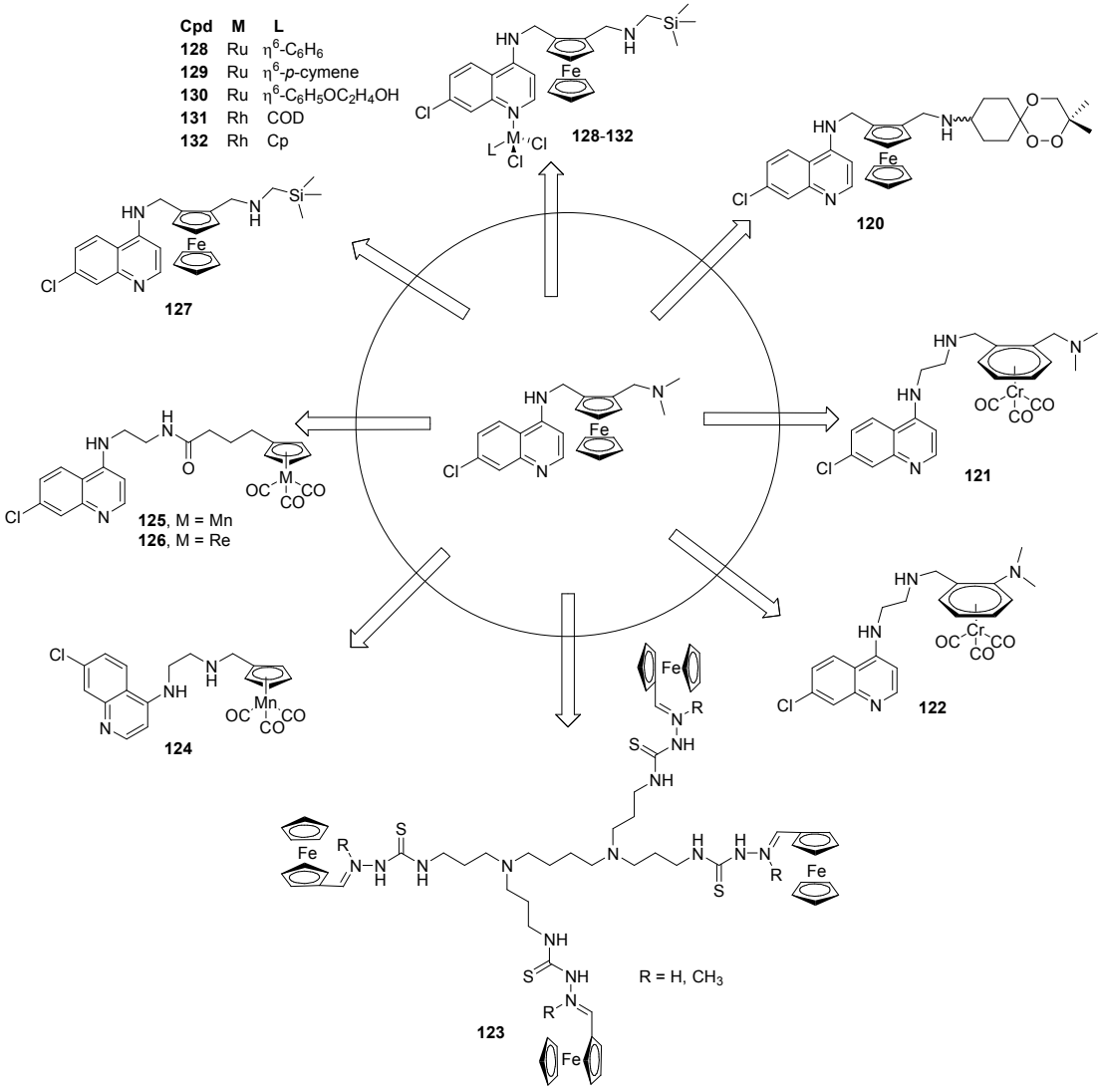


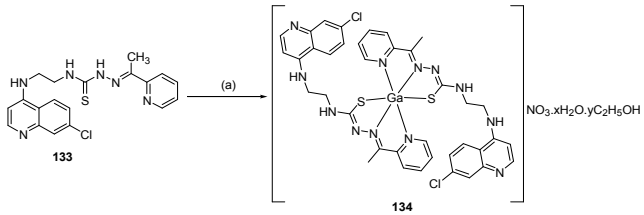
Fig. 17 Some conjugates of ferroquine

After the quintessence of FQ as a potential antimalarial drug, an extensive study was carried out to explore its derivatives with better activity, enhanced ADME properties and structure activity relationship. In a recent study, 4-aminoquinoline, trioxane and ferrocene were covalently linked to form a hybrid (**120**, **Fig. 17**) with the aim to study its antimalarial efficacy. The scaffold revealed potent activity against CQ-resistant *P. falciparum* and was further evaluated *in vivo* against *P. vinckei petteri* infected mice. *In vivo* studies showed a marked decrease in the parasitemia lower than detectable level.[98] Two new "half sandwich" (η^6-arenequinoline) $Cr(CO)_3$ complexes, *viz.* [η^6-*N*-(7-chloroquinolin-4-yl)-*N*′-(2-dimethylaminomethylbenzyl)ethane-1,2-diamine]tricarbonylchromium and [η^6-*N*-(7-chloroquinolin-4-yl)-*N*′-(2-dimethylaminobenzyl)-ethane-1,2-diamine]tricarbonylchromium compounds (**121** and **122**, **Fig. 17**) were prepared and tested *in vitro* for their antiplasmodial efficacy against CQ-susceptible and CQ-resistant strains. The efficacy of complex **122** was two folds higher compared to the organic ligand.[99] In search for new efficient antimalarial complexes, Smith *et al.* synthesized a series of poly(propylene-imine) dendrimers functionalized with ferrocenyl thiosemicarbazones hybridized to the periphery of the molecule (**123**, **Fig. 17**). These metallo-dendrimers exhibited antiplasmodial

activity in the low μM range against W2 strain of *P. falciparum*. Their antiplasmodial potencies were better than non-conjugated ferrocenyl thioesters as well as the free dendritic ligand.[100] Further, organometallic complexes *viz.* cymantrene ($CpMn(CO)_3$) and cyrhetrene ($CpRe(CO)_3$) tethered with 4-aminoquinolines were reported (**124**-**126**, **Fig. 17**) and bio-evaluated for their malarial potency against D10 and Dd2 strains of *P. falciparum*. Low resistance indices (RI) of these complexes indicated that these complexes of manganese and ruthenium may act as lead for further development for new antimalarial compounds.[101]

Smith *et al.* in a recent communication synthesized new carbosilane congener of ferroquine (**127**, **Fig. 17**) by including an organosilicon moiety in the side chain of ferroquine. The carbosilane congener was further utilized for the synthesis of corresponding series of metal complexes of neutral heterometallic ruthenium and rhodium **128**-**132** as shown in **Fig. 17**. All the newly synthesized compounds were evaluated for antiplasmodial activity against the NF54 and Dd2 strain of *P. falciparum*. The presence of the trimethylsilyl group in compound **127** exhibited superior activity (IC_{50} = 7.32 nM) as compared to ferroquine (IC_{50} = 42.65 nM) against NF54 strain. Among synthesized complexes, **130** was the most active with IC_{50} values of 4.86 and 35.91 nM against NF54 and Dd2 strains, respectively.[102]

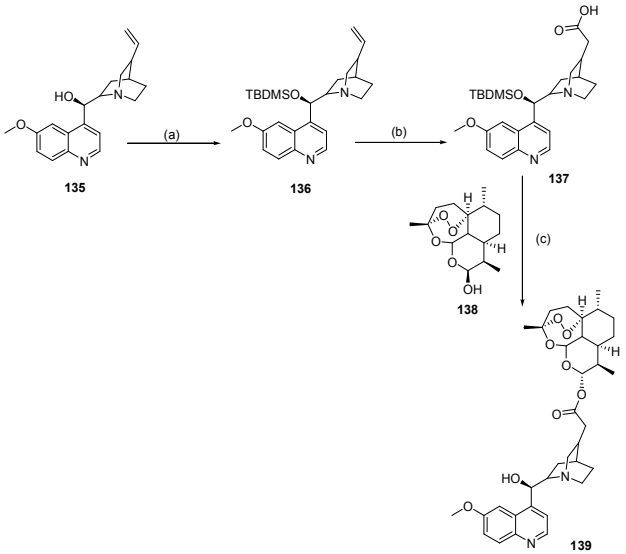
Kumar and co-workers has reported the synthesis of a gallium (III) complex with 7-chloroquinoline thiosemicarbazone as a ligand having antimalarial efficacy against 3D7 strain of *P. falciparum* (**Scheme 18**). The synthesized complex **134** exhibited improved antimalarial activity than lumefantrine on 3D7 isolate of *P. falciparum*. Further, the synthesized complex **134** was evaluated for anti-proliferative activity against different cancer cell lines and complex proved to be 31 times more potent on colon cancer cell line HCT-116, compared with the standard drug etoposide with considerably less cytotoxicity on non-cancerous colon fibroblast, CCD-18Co.[103]



Scheme 18. Reagents and conditions: (a) $Ga(NO)_3.\times H_2O$, ethanol, reflux, 2 h

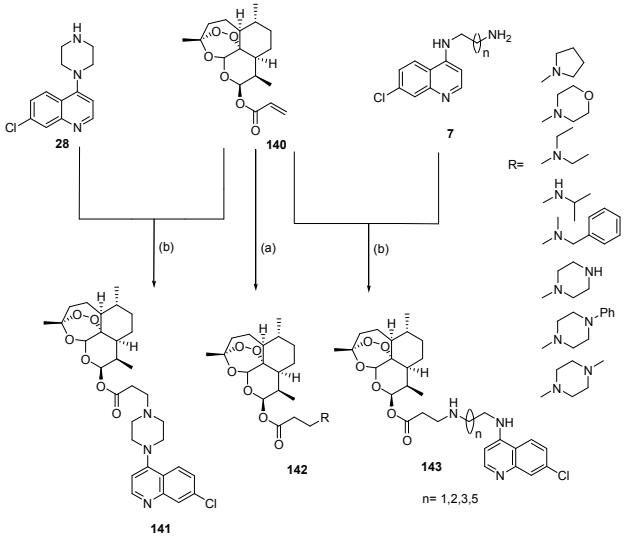
2.10 Arteminsinin and trioxaquine based 4-aminoquinoline conjugates:

The benefits of molecular hybridization over the combination therapy can be well rationalized by the report of Walsh and co-workers involving the synthesis of dihydroartemisinin-quinine hybrids, **139**. The compound was prepared by following a sequence of steps as shown in **Scheme 19** involving an initial protection of quinine with TBDMSiCl with subsequent oxidation to form carboxylic acid and coupling with dihydroartemisinin.[45] The antimalarial efficacy of the synthesized hybrids was determined against CQ-sensitive 3D7 and CQ-resistant FcB1 strains of *P. falciparum*. The conjugate proved to be more potent than either quinine or dihydroartemisinin while a three-fold efficacy was observed over a 1:1 mixture of quinine and dihydroartemisinin.



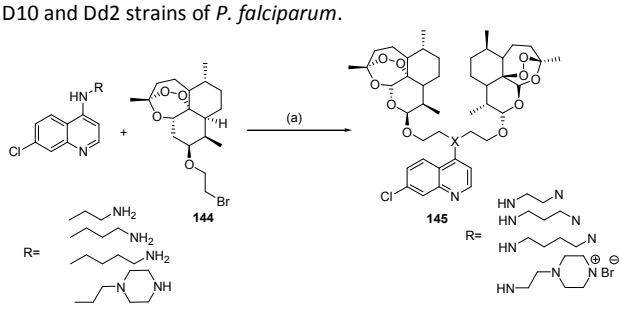
Scheme 19. Reagents and conditions: (a) TBDMSCl, Et_3N, DMAP, DMF, rt; (b) (i) BH_3-THF, diglyme, 0 °C; (ii) $Me_3NO.2H_2O$, 100 °C; (iii) Jones reagent, acetone, rt; (c) (i) 2,6-dichlorobenzoyl chloride, Et_3N, DMAP, DCM, rt; (ii) TBAF, THF, rt

Rationalization of literature has revealed that the variation in the length of alkyl chain between two nitrogen atoms of the 4-diaminoalkyl side chain have a marked influence on the antimalarial efficacy. The compounds with either smaller alkyl chain length (n = 2-4) or with longer alkyl chain length (n = 10-12) retained their activities against CQ-resistant strains, whereas the compounds with chain length (n = 5-8) showed a relative decrease in the activity.[104] Chibale and co-workers have exploited molecular hybridization protocol for the preparation of dihydroartemisinin-7-chloroquinoline conjugates (**141** and **143)** prepared *via* aza-Michael addition reaction along with the analogues of dihydroartemisinin (DHA) derivatives **142** (**Scheme 20**). These were tested for their antimalarial profiles against CQ-sensitive (D10) and CQ-resistant (Dd2) strains of *P. falciparum*. The length of alkyl chain introduced as spacer was chosen between 2-6 carbon atoms for optimal biological activity.[105] The evaluation data revealed a substantial increase in antimalarial efficacy of the hybrids over the precursor **7** or **28** while a decrease in antimalarial efficacy has been observed with the increase in chain length. DHA derivatives, prepared *via* aza-Michael addition with available amines were found to be more effective in the growth inhibition of *P. falciparum* suggestive of the fact that DHA nucleus is mainly responsible for the observed antimalarial activity of the hybrids. The target compound **141** and three dihydroartemisinin derivatives of **125** showed excellent antiplasmodial activity with IC_{50} values of ≤10 nM against both D10 and Dd2 strains of *P. falciparum* and low cytotoxicity against human cell lines (HeLa cells).



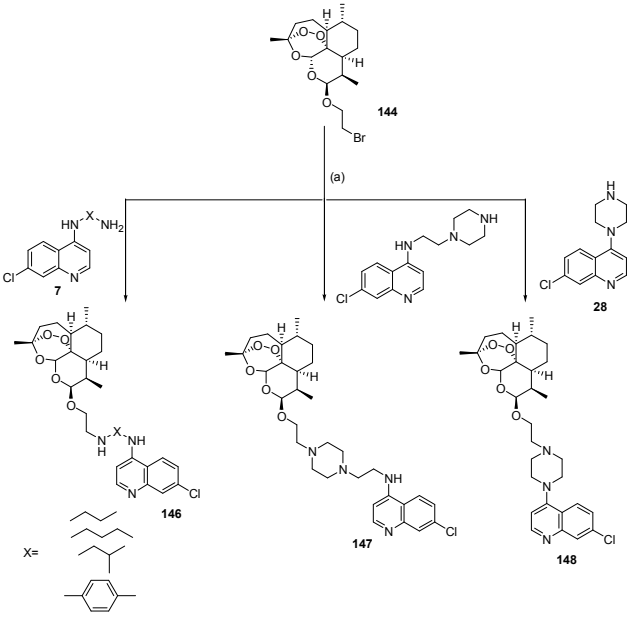
Scheme 20: Reagents and conditions: (a) Appropriate amine, DBU, CH_3CN, rt, 12h; (b) DBU, DMF, N_2, rt, 12h

N'Da further extended his work on the synthesis of artemisinin-quinoline and their hybrid-dimers from dihydroartemisinin and aminoquinolines (**Scheme 21**).[106] The synthesized hybrids **145** obtained as β-isomers were assessed for their antiplasmodial activity against CQ-sensitive and -resistant strains of *P. falciparum* along with its cytotoxicity profiles against mammalian cell-line. Although, none of the hybrids was found to be more potent than DHA, two were more active than CQ. Hybrid-dimer having an optimum chain length of three carbon atom displayed excellent antimalarial potency, with IC_{50} values of 5.31 and 28.43 nM against D10 and Dd2 strains of *P. falciparum*.



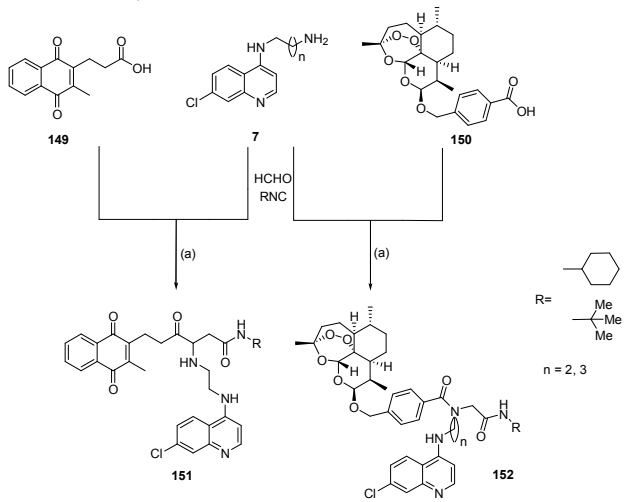
Scheme 21. Reagents and conditions: (a) DMF, 90-110 °C, 6-8 h

N'Da with his co-workers has explored the synthesis of dihydroartemisinin-4-aminoquinoline conjugates (**146**, **147** and **148**) with their subsequent conversion to oxalate salts (**Scheme 22**). *In vitro* antimalarial activity was tested against CQ-sensitive D10 and -resistant Dd2 strains of *P. falciparum* while cytotoxicity was determined against mammalian Chinese Hamster Ovarian (CHO) *via* 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazoliumbromide (MTT)-assay.[107] Most of the synthesized conjugates showed comparable to higher activity to that of standard drug CQ with IC_{50s} ranging from 17.2 to 38.9 nM. The oxalate salts proved to be seven times more potent than the standard drug CQ against the CQ-resistant strain of *P. falciparium*.



Scheme 22: Reagents and conditions: (a) DMF, 70-80 °C, 4-6 h

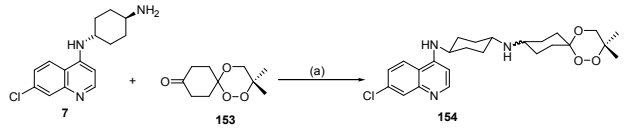
In 2011, Chibale *et al.* have extended the synthesis of artemisinin-4-aminoquinoline conjugates (**151**, **152**) *via* Ugi four-component condensation (Ugi-4CR) reaction (**Scheme 23**)[108] using paraformaldehyde as aldehyde component, 7-chloro-4-diaminoalkylquinoline as the amine input, artelinic acid/1,4-napthoquinone acid as acid input, and cyclohexyl-or *tert*-butyl isocyanide as the isocyanide input. Antiplasmodial studies of the synthesized conjugates showed comparable potency against CQ-resistant K1 and CQ-sensitive D10 strains of *P. falciparum*. Mechanistically, the conjugates exhibited potent inhibitory activities of β-hematin formation and appeared to inhibit endocytosis as evident by the decrease in the number of transport vesicles in the parasite.



Scheme 23. Reagents and conditions: (a) succinic acid, silver nitrate, ammonium persulfate, 30% aq. CH_3NH_2, 65-70 °C, 3 h

Meunier *et al.* reported the synthesis of trioxaquines **154** as potential antimalarials by following the synthetic protocol as

depicted in **Scheme 24**.[109] Compound trioxaquine (PA1103/SAR116242) **154**, obtained from this study proved to be highly active against several sensitive and resistant strains of *P. falciparum* at nM concentrations. It has also shown activity on multidrug-resistant strains attained from fresh patient isolates in Gabon along with good drug profiles *viz.* absorption, metabolism and safety parameters. Efficacy of this conjugate *via* oral route is very high with total curing of mice infected with CQ-sensitive and -resistant strains of *Plasmodium* at 26-32 mg/kg.



Scheme 24. Reagents and conditions: (a) $NaBH_3CN$, MeOH, HCl/*i*-PrOH

The selection of trioxaquine (PA1103/SAR116242) for antimalarial drug development strongly confirms the role of molecular hybridization in furnishing molecules with satisfactory pharmacological and safety profiles to allow regulatory drug development. Guided by similar rationale, O'Neill and co-workers reported the synthesis of semi-synthetic trioxaquines and trioxolaquines in excellent yields and assessed their antimalarial activities against CQ-sensitive (3D7) and -resistant (K1) strain of *P. falciparum*. Compounds of both the series showed activity in low nM range with some of the analogues being more active than artemisinin. Structure of two of the most active compounds **155** and **156** of the series is as shown in **Fig. 18**.[110]

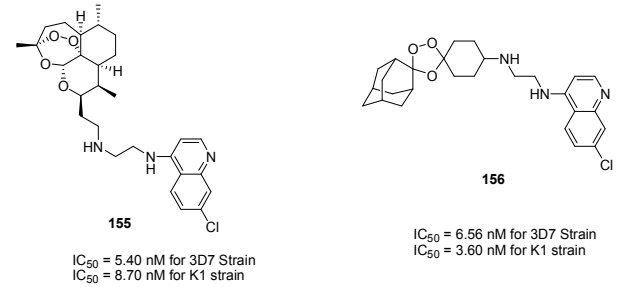
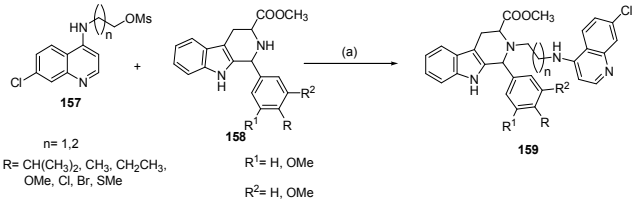


Fig. 18 Most potent 1,2,4-trioxaquines and 1,2,4-trioxolaquines conjugates

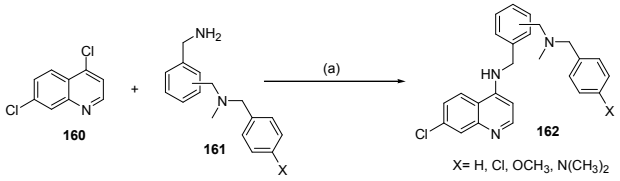
2.11 Miscellaneous antimalarial 4-aminoquinoline conjugates:

A library of tetrahydro-1*H*-β-carbolines-4-aminoquinoline hybrids **159** were identified as a novel class of highly potent antimalarial agents by Chauhan *et al.* using molecular hybridization approach with MIC values ranging from 0.05 to 19.08 μM against CQ-sensitive strain NF-54 of *P. falciparum* (**Scheme 25**).[111] Most potent of the synthesized conjugate was found to have seven folds more activity than the standard drug CQ and therefore warrants the development of these hybrids in antimalarial chemotherapy.



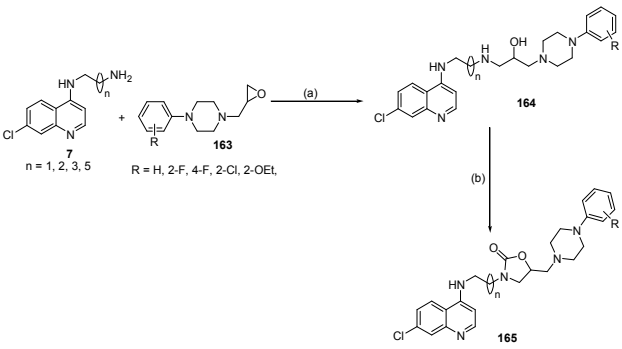
Scheme 25. Reagents and conditions: (a) DMF, 120 °C, high pressure

In 2011, Egan and collaborators proposed the synthesis of dibemethin-coupled 4-aminoquinolines **162** as potential antimalarial agents with a view to obtain novel hybrids capable of inhibition of hemozoin formation in the digestive vacuole of parasite (**Scheme 26**).[112] These hybrids were equally potent against cultured CQ-sensitive (D10) and CQ-resistant (K1) *P. falciparum*. Several compounds exhibited *in vitro* antimalarial potency below 100 nM against both strains of the parasite while none of compound exhibited cross-resistance with the standard drug CQ. The compounds with promising *in vitro* activity and non-cytotoxicity against mammalian cell line were screened for their *in vivo* activity against *P. berghei*. The active compounds exhibited significant *in vivo* activity and reduces parasitemia by over 99%. SAR studies showed marked dependence of activity on the site of attachment of 4-aminoquinoline with side chain of dibemethin as well as the substituent on the terminal phenyl ring of dibemethin. Researchers further investigated the interaction of this series of compounds with CQ transport by making use of Xenopus oocyte expression system which directly measures CQ transport by *P. falciparum* CQ-resistance transporter (PfCRT) and its inhibition with potential resistance-reversers or reversed-CQ compounds. The results of this study confirmed 4-aminoquinoline-dibemethin hybrids as the first example of reversed-CQ antimalarials with capability to inhibit both PfCRT and hemozoin formation.



Scheme 26. Reagents and conditions: (a) Anhyd. *N*-methyl-2-pyrrolidine, K_2CO_3, Et_3N

Recently, Chibale *et al.* reported the design and synthesis of novel β-amino alcohol derivatives of 4-aminoquinoline **164** with an extension to 4-aminoquinoline-oxazolidinone conjugates **165** (**Scheme 27**).[113] *In vitro* antimalarial activity was evaluated against a CQ-sensitive (3D7) and CQ-resistant (K1) strains of *P. falciparum*. Few of the β-amino alcohol analogues were more active compared to CQ against CQ-sensitive strain while the potency decreased considerably against CQ-resistant strain revealing cross resistance of these conjugates with CQ. The conversion of β-amino alcohol to oxazolidinones by reacting with tris-phosgene resulted in considerable decrease in activities of the synthesized conjugates against both CQ-sensitive and CQ-resistant strains.



Scheme 27. Reagents and conditions: (a) MeOH, 55 °C; (b) tris-phosgene, DCM, 0 °C-rt

Chauhan *et al.* have synthesized sixteen new 4-aminoquinoline analogues by modifying its side chain with an extension towards the synthesis of quinoline-acridine conjugates and tested their *in vitro* antiplasmodial activity against NF 54 strain of *P. falciparum*.[114] Among the tested compounds, the compound **166** (MIC = 0.125 μg/ml) was nearly as active as CQ (MIC = 0.125 μg/ml) while another (**167**) (MIC = 0.031 μg/ml) exhibited four-fold more potency than CQ. Three of the selected test compounds **166**, **167** and **168** (**Fig. 19**) with good *in vitro* antiplasmodial potency were chosen for *in vivo* screening in Swiss mice infected with CQ-resistant N-67 strain of *P. yoelii*. Compound **167** showed curative response to all treated Swiss mice at doses 50 mg/kg and 25 mg/kg for 4 days given *via* intra-peritoneal route. The compound was orally active at the dose of 100 mg/kg and has proved the importance of exploring the 4-aminoquinoline class for the discovery of new antimalarial agents.

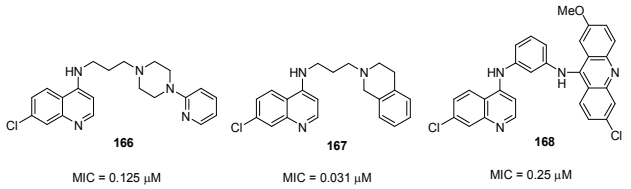


Fig. 19 Most potent compounds among the series

Hybrid design strategy was further exemplified by Whitelock and co-workers in the synthesis of CQ-astemizole (non-sedating H1 antagonist) conjugates **170** along with their antiplasmodial evaluation against K1 strain of *P. falciparum* (**Fig. 20**).[115] Most of the conjugates were three to folds more potent than CQ with the most potent of the tested compound exhibiting an IC_{50} of 23 nM. Importantly, the compounds displayed more than 100 times selectivity for antiparasitic activity over cell-based cytotoxicity. Based on the preliminary *in vitro P. falciparum* activity, two potent compounds were further screened for *in vivo* studies on *P. berghei* mouse malaria model.

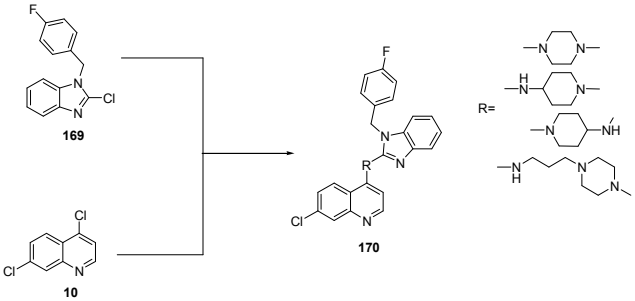


Fig. 20 General strategy of 7-Chloroquinoline-Astemizole based hybrids

Santos and their colleagues have synthesized molecular hybrids of commercially available squarates with the antimalarial drugs, chloroquine (CQ) and primaquine (PM) with improved antiplasmodial activity compared with the squarates.[116] Three of the synthesized conjugates containing two 7-chloroquinoline moieties were two fold more active than the standard drug, CQ with IC_{50} values ranging from 0.095-0.20 μM against CQ-resistant W2 strain of *P. falciparum* (**Fig. 21**). Length of the carbon chain introduced as linker between squarates and quinoline scaffolds has shown to have a major impact on the observed activity profiles with the activity generally increasing with the increase in chain length.

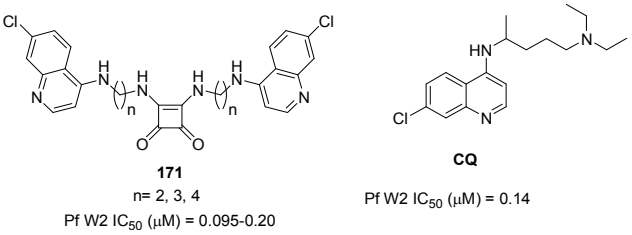


Fig. 21 Most potent 4-aminoquinoline-squarate hybrids and CQ

Vacuolar plasmepsins, pepsin-like aspartic proteases present in different species of parasite *Plasmodium*, are recognized as promising targets for the enlargement of new antimalarial agents.[117] It comprises of plasmepsins 1 (PLM 1), plasmepsins 2 (PLM 2), plasmepsins 4 (PLM 4) and a histo-aspartic protease (HAP) and play an vital role in the survival of the parasite. These enzymes are contained in the food vacuole of parasite and are directly involved in the degradation of human haemoglobin during the erythrocytic stage. Statin-based molecules are shown to be potent inhibitors of PLMs with however, restricted effectiveness in killing the parasite (IC_{50} range 2-20 μM).[117] In 2012, Romeo and co-workers, in an attempt to circumvent the limitation associated with statin-based molecules have synthesized its hybrids with 4-aminoquinoline **172**.[118] It was considered that since both of these molecules interfere with the haemoglobin catabolism process, the resulting hybrid will have an improvement in overall antimalarial potency.[119] Two of the 4-aminoquinoline-statin hybrids **172a** and **172b** were more potent than CQ against CQ-resistant strain (**Fig. 22**) with good selectivity against cathepsin D. It was concluded from β-hematin inhibitory assay that 7-chloro group in the quinoline ring is obligatory for the antiplasmodial efficacy of the synthesized conjugates.

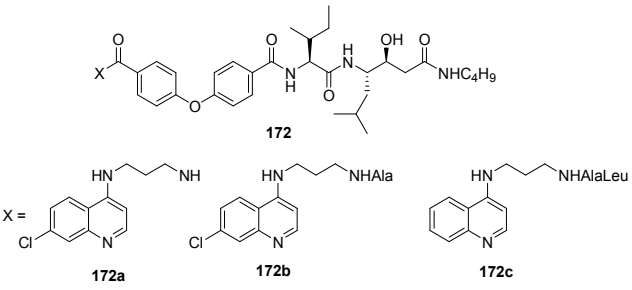
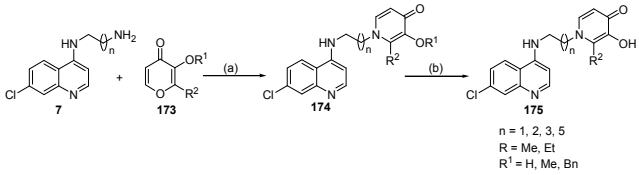


Fig. 22 Most potent 4-aminoquinoline-statin hybrids **171a**, **171b**, **171c**

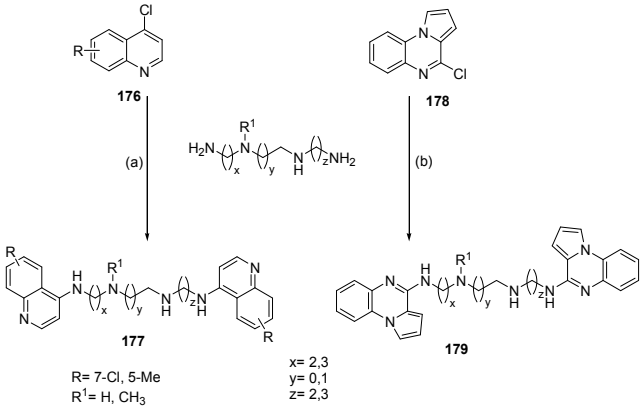
Chibale and co-workers has reported the preparation of 4-aminoquinoline-3-hydroxypyridin-4-one conjugates on the basis of additive *in vitro* combination of *N*-alkyl-3-hydroxypyridin-4-one with chloroquine (CQ) (**Scheme 28**). All the synthesized conjugates were assessed *in vitro* against CQ-resistant (K1 and W2) and -sensitive (3D7) strains of *P. falciparum* along with β-hematin formation studies.[120] Most of conjugates were shown to have superior β-hematin inhibition activity to that of CQ and a strong correlation

was observed between antimalarial profiles and inhibition of β-hematin formation. Among the synthesized conjugates, three potent compounds with longer alkyl chain (n = 3, 5) against K1, 3D7, and W2, respectively, having IC_{50s} of **175c** (0.13, 0.004, and 0.1 μM); **175d** (0.08, 0.01, and 0.02 μM); and **174g** (0.07, 0.03, and 0.08 μM). Cytotoxicity study on mammalian cell line showed that most of the conjugates screened were non-cytotoxic and far less toxic than podophyllotoxin (POD).



Scheme 28. Reagents and conditions: (a) 50% aq EtOH, pH 13 (2 M NaOH), 90-120 °C, 18-24 h; (b) H_2, Pd/C, 2 M ethanolic HCl, 4 atm or 1:2:3 H_2O/EtOH/HCl, 74 °C, 24 h

Another attempt to explore the antimalarial potential of bis-quinolines was made by N'Da and co-workers by exploring the synthesis of a series of bis-quinolines and bis-pyrrolo[1,2a]quinoxalines containing various polyamine linkers along with the determination of their aqueous solubility and distribution coefficients (**Scheme 29**).[121] Antimalarial potency of these compounds was assessed against CQ-sensitive (D10) and CQ-resistant (Dd2) strains of *P. falciparum* besides the growth inhibitory studies against a panel of cancer cell lines. Bis-quinolines showed a general increase in antimalarial activity with the increase in protonation sites given by polyamine linkers that could help to overcome CQ-resistance. The 7-chloro-substituted hybrids have shown to possess more potency compared to either 5-methyl quinoline or pyrrolo[1,2a]quinoxaline conjugates. Among bis-quinoline, triethylene-tetramine and *N*,*N*-bis(3-aminopropyl)ethylene-diamine linkers, were found to be the most potent of all the synthesized conjugates.



Scheme 29. Reagents and conditions: (a) 135-145 °C, 16-20 h; (b) K_2CO_3, DMF, 135-145 °C, 16 h

Chibale *et al.* has explored molecular hybridization paradigm for the synthesis of three groups of hybrid molecules by covalent fusion of azidothymidine (AZT) with dihydroartemisinin (DHA), a tetraoxane or a 4-aminoquinoline derivative that target *P. falciparum* and HIV simultaneously. All synthesized compounds were tested for their inhibitory activity against pseudotyped HIV-1, CQ-sensitive (3D7) and CQ-resistant (Dd2) strains of *P. falciparum* at three different concentrations (50, 5 and 1 μM) along with cytotoxicity assessment against HeLa cells. Among tested conjugates, potent CQ-AZT conjugate (**180**, **Fig. 23**) exhibited IC_{50} values of 0.38 and 0.08 μM (more potent than CQ and tetraoxane) against 3D7 and Dd2 strains of *P. falciparum*. This compound also exhibited enhanced inhibitory activity (IC_{50} value of 0.9 μM) against pseudotyped HIV-1 with selective index of 31.8. However, higher cytotoxicty in HeLa cells discontinued its further testing.[122]

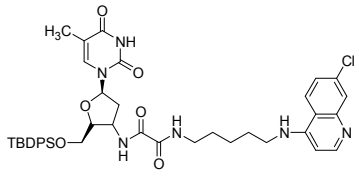


Fig. 23: Most potent CQ-AZT compound **180**

In a recent communication, Rao *et al.* reported the synthesis of a series of 4-aminoquinoline-4*H*-chromene conjugates along with their antimalarial evaluation against two *P. falciparum* strains namely 3D7 (CQ-sensitive) and K1 (CQ-resistant).[123] All synthesized conjugates possess activity in μM range with compounds **181** having piperazine linkage and nitro substituent and **182** with azapane linkage and chloro substituent at C-6 position of 4*H*-chromene showed comparable activity to that of CQ (**Fig. 24**). Further, molecular docking suggested that these conjugates exhibited strong binding affinity with *P. falciparum* Lactate dehydrogenase (PfLDH) and are acting as potent inhibitors of the parasite specific glycolytic pathway.

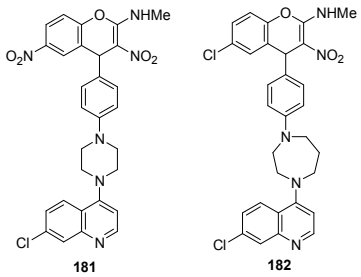


Fig. 24: Most potent 7-chloroquinoline-4*H*-chromene conjugates **181** and **182**

Chibale *et al.* has recently disclosed the synthesis of 4-aminoquinoline-benzoxazole conjugates and screened for their antimalarial efficacy against K1 (multidrug resistant) and NF54 (sensitive) strains of the parasite *P. falciparum*.[124] Antiplasmodial activities of synthesized compounds exhibited good structure-activity relationship which led to the recognition of highly promising conjugates having IC_{50}s in the nM range against both K1 and NF54 strains of *P. falciparum*. Further, the synthesized conjugates showed good *in vitro* microsomal metabolic stability along with desirable *in vivo* pharmacokinetic studies. Four most promising conjugates (**183**, **184**, **185** and **186**, **Fig. 25**) were subjected to *in vivo* antimalarial studies against *P. berghei* infected mice.

Compound **183** showed good *in vivo* oral efficacy and completely cured the treated mice at a low multiple dose of 4-10 mg/kg. Mechanistic studies revealed the inhibition of hemozoin formation as one of the probable mechanisms of action for these compounds. Further, β-haematin inhibition studies confirmed the presence of quinoline nucleus for antiplasmodial activity as non-benzoxazole intermediates did not show any inhibitory activity even at highest tested concentration.

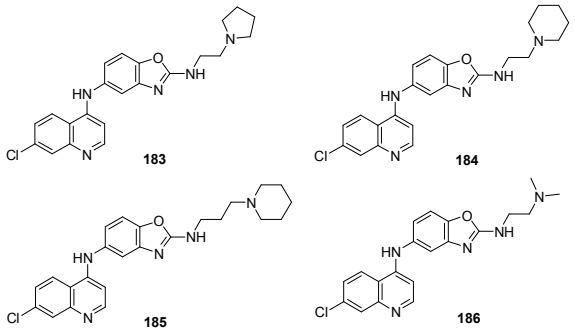
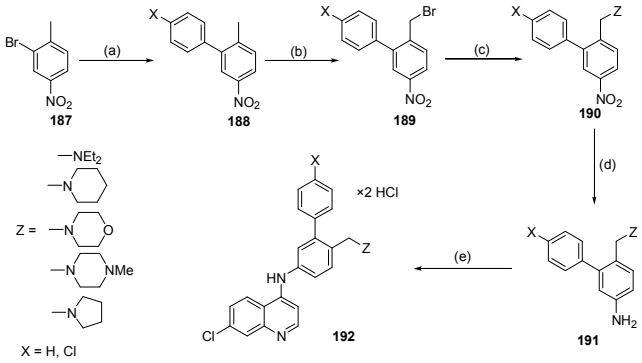


Fig. 25: Most potent 7-chloroquinoline-benzoxazole conjugates **183**, **184**, **185** and **186**

Lopez and co-workers in a recent communication, has shown the synthesis of 3'-dehydroxy-isotebuquine analogs of 4-aminoquinoline **192** *via* five step synthetic sequence in good to excellent yields as shown in **Scheme 30**. The synthesized conjugates were assessed for their β-hematin inhibitory activity. Among eight of the synthesized conjugates, six compounds were shown to have greater than 97 % IHF value (% inhibition of haemozoin formation). These potent compounds were further tested for their *in vivo* antimalarials activity in mice infected with *P. berghei* ANKA CQ-susceptible strain with three of the conjugates displaying *in vivo* antimalarial efficacy comparable to that of CQ.[125]



Scheme 30. Reagents and conditions: (a) phenylboronic acid, toluene, $Pd(PPh_3)_4$ (5%), K_2CO_3, EtOH:H_2O (1:1), 100 °C, 24 h; (b) NBS, CCl_4, $(PhCO)_2O_2$, light, reflux, 24 h; (c) dialkylamine, toluene, reflux, 6 h; (d) Sn/HCl, 70 °C, 2 h; (e) 4,7-dichloroquinoline, EtOH, HCl (cat.), reflux, 6 h

3. Conclusions

The development of new drugs with improved physicochemical profiles, lack of toxicity along with synthetic selectivity and economic accessibility represents a big challenge for the pharmaceutical sector and warrants continuous efforts. The present review established the attention of organic medicinal chemists in the field of 4-aminoquinoline-hybridization towards the synthesis of new molecular frameworks for averting and delaying the emergence of drug resistance along with improvement in efficacy. The validation of molecular hybridization, however, is crucial and the benefit of the hybrid over the separate pharmacophores or their 1:1 combination should be confirmed.

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