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# Design, Synthesis and *in Vitro* Antiplasmodial Activity of Some Bisquinolines against Chloroquine Resistant Strain

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**Short Running Title**: Antiplasmodial Activity of Some Bisquinolines.

***Keywords:*** 4-Aminoquinoline; Antiplasmodial activity; Chloroquine; *Plasmodium falciparum*; Chloroquine sensitive strain; Chloroquine resistant strain.

***Abbreviations:*** CQ, chloroquine; DCC, *N*,*N*-dicyclohexylcarbodiimide; HOBt, hydroxybenzotriazole; DMF, dimethylformamide.

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**Abstract:**

A series of novel bisquinoline compounds comprising, $N^1$-(7-chloroquinolin-4-yl) ethane-1,2-diamine and 7-chloro-*N*-(2-(piperazin-1-yl)ethyl)quinolin-4-amine connected with 7-chloro-4-aminoquinoline containing various amino acids is described. We have bio-evaluated the compounds against both chloroquine-sensitive (3D7) and chloroquine-resistant (K1) strains of *Plasmodium falciparum in vitro*. Among the series, compounds **4** and **7** exhibited 1.8 and 10.6 fold superior activity as compared to chloroquine (CQ) ($IC_{50}$ = 0.255±0.049 µM) against the K1 strain with $IC_{50}$ values 0.137±0.014 and 0.026±0.007 µM respectively. Furthermore, compound **7** also displayed promising activity against the 3D7 strain ($IC_{50}$ = 0.024±0.003 µM) of *P. falciparum* when compared to CQ. All the compounds in the series displayed resistance factor between 0.57 and 4.71 as against 51 for CQ. These results suggest that bisquinolines can be explored for further development as new antimalarial agents active against chloroquine resistant *P. falciparum.*

## 1. Introduction

Even after several decades of CQ (Figure 1) discovery, it is still considered as a mainstream drug for the control and eradication of malaria. The immense success of CQ is due to its unique features like limited host toxicity, less side effects and affordability [1]. However, in recent years efficacy of CQ and other 4-aminoquinoline analogues has steadily declined due to emergence of drug resistant parasites in the endemic regions [2]. Therefore, it is a clear indication for researchers to discover new chemical entities with high therapeutic efficacy against chloroquine resistant (CQ-R) parasite. During the literature survey it was

found that a number of bisquinolines exhibited potential antiplasmodial activity particularly against CQ-R parasite [3]. The early examples of such agents include bis(quinolyl)piperazines such as piperaquine (Figure 1) which was first synthesized in the year 1960. Efficacy, bioavailability and good pharmacokinetic properties led this molecule to therapeutic application. Other drug leads such as dichloroquinazine (12,278RP) and the most promising molecule Ro 47-7737 (Figure 1) were found to be potent, but could not be pursued as drug candidates due to toxicity. The available literature data suggest that bisquinolines are less likely to be affected by the efflux mechanism operating in CQ-R *P. falciparum* [4-5].

**Figure 1 Structures of some antimalarial agents**

Subsequently, Cowman *et al.* described the synthesis of a novel series of bisquinolinemethanols (Figure 2 A), which exhibited better activity than CQ against CQ-R parasite, but because of toxicity, these molecules were not pursued [6]. Later, Vennerstrom and co-workers synthesized a series of bisquinolines (Figure 2 B) with nitrogen and oxygen containing alkane linkers, which were found to be more potent against both CQ-sensitive (D6) as well as CQ-resistant (W2) strains of *P. falciparum* in the *in vitro* studies [7]. Further, Raynes *et al.* explored two series of bisquinolines (Figure 2 C) having bisamide linkage at 6 and 8 positions of the quinoline nucleus. These analogues were found to have moderate activity against CQ-sensitive strain (D10) and good activity against CQ-resistant strains (FAC8 and K1) of *P. falciparum*. The activity profile of these bisquinolines suggested that the position of attachment and length of the linker chain markedly affected the activity [8]. Furthermore, Girault's group synthesised bis, tris and tetraquinolines (Figure 2 D) by introducing proton accepting and/or substitution sites, in order to maintain both steric hindrance and conformational flexibility of quinoline nucleus, but they were found to be less potent than their linear counterparts [9]. More recently, Tomar *et al*. have synthesized a series

of substituted bisquinolines (Figure 2 E, R=$CH_3$, H; $R^1$=H, $CH_3$, $C_2H_5$) having excellent *in vitro* activity against CQ-S parasite [10].

**Figure 2 Structures of some bisquinoline antimalarials.**

Previously from our group, we have reported a series of CQ analogues obtained by modifying amine side chain derived from various amino acids (Figure 3 a). These CQ analogues showed good activity ($IC_{50}$ ranging between 11.51 and >500 nM) against CQ-sensitive (3D7) strain and very good activity ($IC_{50}$ ranging between 55.29 and >500 nM) against CQ-resistant (K1) strain of *P. falciparum* [11]. Recently, Kondaparla *et al.* have synthesized a series of side chain modified 4-aminoquinolines by connecting $N^1$-(7-chloroquinolin-4-yl)ethane-1,2-diamine to different amino acids (Gly, Ala, Val, Leu, Ile, Phe, Trp and Met) *via* peptide (-CO-NH)- linkage (Figure 3 b). These molecules were found to have moderate activity ($IC_{50}$ ranging between 0.015±0.002 and 3.22±0.041 µM) against 3D7 strain and promising activity ($IC_{50}$ ranging between 0.17±0.002 and >1.0±0.045 µM) against K1 strain of *P. falciparum*. The results indicated that the hydrophobicity of the amino acid side chain and pendant amine group is essential for antiplasmodial activity of 4-aminoquinolines [12].

Based on the above information, we thought it appropriate to introduce amide bond with heterocyclic ring system (7-chloro-4-aminoquinoline) on lateral side chain of 4-aminoquinoline as it will provide lipophilic nature to the molecules and could give compounds with improved antimalarial activity. The quinoline nucleus was selected based on the observation that it is a biologically privileged scaffold and well tolerated in human subjects [13]. In continuation of the antimalarial drug discovery programme in our group

herein we report the efficient synthesis of new bis(4-aminoquinoline) analogues (Figure 3 c) (scheme 1) and their antiplasmodial activity.

**Figure 3 Some lead molecules of 4-aminoquinoline derived antimalarials developed from this laboratory.**

## 2. Results and Discussion

### 2.1 *Chemistry*

The desired bisquinolines **4-15** were synthesized via coupling of $N^1$-(7-chloroquinolin-4-yl)ethane-1,2-diamine (**1)** and 7-chloro-*N*-(2-(piperazin-1-yl)ethyl)quinolin-4-amine (**3)** with respective intermediates **2a-2h** using (DCC/HOBt) as a coupling reagent (scheme 1). Further, synthesis of intermediates **1** and **3** were achieved by the nucleophilic aromatic substitution reaction (SNAr) executed on 4,7-dichloroquinoline using 1,2-diaminoethane and 2-aminoethylpiperazine as reactive precursors respectively, in good yields. However, when the same reaction was performed for the preparation of intermediates **2a-2h**, the desired products could not be obtained. Subsequently, we have achieved the synthesis of required intermediates **2a-2h** by performing the reaction in phenol at 160 °C. The final compounds (**4-15**) were characterized by $^{1}$H NMR, $^{13}$C NMR, ESI-MS and HRMS respectively.

**Scheme 1 Synthesis of bisquinoline antimalarials.**

### 2.2 *Antiplasmodial (P. falciparum) Activity*

The bisquinolines **4-15** have the $IC_{50}$ values ranging between 0.024±0.003 and 1.69±0.045 μM against chloroquine sensitive (CQ-S) and 0.026±0.007 to 2.09±0.049 μM against chloroquine resistant (CQ-R) strains of *P. falciparum in vitro* and the results are shown in Table 1. From the *in vitro* results, it is clear that bisquinolines constructed with alkylamine linker were more potent than the corresponding piperazine linker analogues. However, bisquinolines **4**, **5** and **8-15** displayed poor activity against the CQ-S strain with $IC_{50}$ values

ranging between 0.155±0.017 and 1.69±0.045μM as compared to CQ ($IC_{50}$ = 0.005±0.002 μM). On the other hand compound **4** showed superior antiplasmodial activity against the CQ-R strain ($IC_{50}$ = 0.137±0.014 μM) when compared to CQ. Moreover, compounds **9** and **11** exhibited activity comparable to CQ against the CQ-R strain of *P. falciparum* with $IC_{50}$ values 0.304±0.028 μM and 0.355±0.037 μM respectively. Among all the bisquinolines, compound **7** with an isobutyl group in the side chain displayed good inhibition of parasite growth against the CQ-S strain ($IC_{50}$ = 0.024±0.003 μM). It is important to mention here that this compound also showed 10.6 fold superior activity against the CQ-R strain of *P. falciparum* with $IC_{50}$ = 0.026±0.007 μM as compared to CQ ($IC_{50}$ = 0.255±0.049 μM). By comparison, compound **13** containing a piperazine linker exhibited very poor inhibition of parasite growth ($IC_{50}$ = 1.69±0.045 μM (3D7), 2.09±0.049 μM (K1) against both strains of *P. falciparum*. It may be appropriate to mention that some of our compounds have exhibited very low resistance factor values ranging from 0.57-4.71 than the reference drug CQ which is having resistance factor value 51. From the *in vitro* data it is clear that there is a correlation between length of the bisquinoline heteroalkane linker and the antiplasmodial activity, this is in agreement with the published report from Vennerstrom *et al.* [7].

**2.3 *Discussion***

According to the obtained *in vitro* results, it has been observed that nature of the linker plays a vital role on the inhibition of parasite growth against both CQ-S and CQ-R strains of *P. falciparum.* In general, bisquinoline analogues **4-11** containing alkylamine linkers were more potent than piperazine linker bisquinolines **12-15** (**Table 1; eg 7 vs 13, 9 vs 14** and **10 vs 15)** and this may be due to increased rigidity and decreased flexibility of the piperazine moiety. One exception is that of **6** compared with compound **12,** with the piperazine linker compound showing activity against both strains ($IC_{50}$ =0.884±0.046 μM (3D7), 0.510±0.036 μM (K1)). Compound **4** having simple hydrogen as its side chain showed

moderate activity against the 3D7 strain with $IC_{50}$ value 0.155±0.017 μM, and slightly better activity than CQ when screened against the CQ-R strain of *P. falciparum*. Further, in the case of alanine analogue **5** the activity was decreased significantly against both strains ($IC_{50}$ = 0.250±0.021 μM (3D7), 0.923±0.032 μM (K1) of *P. falciparum*. When the amino acid side chain is changed to methionine, this afforded compound **11** with higher activity against the 3D7 strain ($IC_{50}$ = 0.386±0.033 μM) and activity comparable to CQ when screened against the K1 strain ($IC_{50}$ = 0.355±0.037 μM) of *P. falciparum*. Among all bisquinolines of the series, compound **7** with an isobutyl group in the side chain exhibited 10.6 fold superior activity against the CQ-R strain ($IC_{50}$ = 0.026±0.007 μM) and promising activity against the CQ-S strain ($IC_{50}$ = 0.024±0.003 μM) as compared to CQ. As already discussed, the activity was reduced drastically against both strains (3D7 & K1) when rigid aminoethylpiperazine moiety was incorporated instead of the flexible alkanediamine linker as in compound **13**.

The structure activity relationship (SAR) studies of bis(4-aminoquinolines) suggested that 7-chloro group and quinoline scaffolds are most favourable for antiplasmodial activity (Figure 4). The length of the carbon chain linker in the side chain of bisquinoline plays a key role on the antiplasmodial activity against chloroquine resistant strain of *P. falciparum*. It is evident from the results that bisquinolines containing short linker such as 2 carbon chain (ethane-1,2-diamine) were appropriate for the activity. However, bisquinolines containing longer and conformationally rigid linker such as 1-(2-aminoethyl) piperazine have shown poor activity. Furthermore, presence of an isobutyl group in the linker potentiates the biological activity (Figure 4).

**Figure 4 SAR of the synthesized potent active bisquinoline**.

### 2.4 *In Vitro cytotoxicity*

The cytotoxicity of synthesized bisquinolines **4-15** was determined by the standard protocol, MTT assay against VERO cell line (Table 1). All bisquinolines displayed the selectivity index (SI) values ranging from 20.50 to 1011.71. The compound **7** showed good selectivity index (SI) 1011.71 (Table 1) and equally good parasite growth inhibition against the CQ-R strain of *P. falciparum*., The remaining compounds in the series showed less cytotoxic effect with fairly considerable selectivity index (SI) and moderate antiplasmodial activity.

**Table 1 Antiplasmodial activity of bisquinolines (4-15) against *P. falciparum in Vitro.***

## 3. Conclusion

In conclusion, we have synthesized a new series of bisquinolines and screened for the antiplasmodial activity against both CQ-S and CQ-R strains of *P. falciparum*. From the activity data, it may be inferred that bisquinolines with alkylamine linker were more potent than the corresponding piperazine linker analogues. Further, compound **7** exhibited 10.6 fold superior antiplasmodial activity against the CQ-R strain and promising activity against the CQ-S strain of *P. falciparum*. Furthermore, almost all the compounds in the series showed resistance factor values much lower than CQ. From this exploration, we have identified the lead compound **7 [**(*S*)-2-(7-chloroquinolin-4-ylamino)-*N*-(2-(7-chloroquinolin-4-ylamino) ethyl)-4-methylpentanamide] having potent activity against both CQ-S and CQ-R strains of *P. falciparum* .

**Acknowledgment**

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**Conflicts of Interest**

The authors have no conflict of interest to declare

**Supporting Information available (Appendix S1):** Biological assay & materials and methods of synthesized compounds.

## Figure Legends

**Figure 1** Structures of some antimalarial agents

**Figure 2** Structures of some bisquinoline antimalarials.

**Figure 3** Some lead molecules of 4-aminoquinoline derived antimalarials developed from this laboratory.

**Figure 4** SAR of the synthesized potent active bisquinoline.

**Scheme 1** Synthesis of bisquinoline antimalarials.

**Table 1** Antiplasmodial activity of bisquinolines (4-15) against *P. falciparum in Vitro.*

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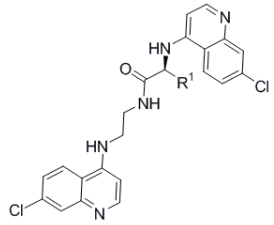
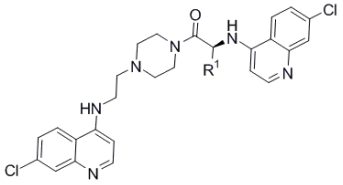
**Table 1** Antiplasmodial activity of bisquinolines (**4-15**) against *P. falciparum in Vitro.*

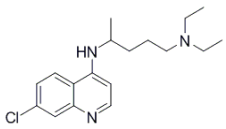


**4-11**



**12-15**

| Compd. no | $R^1$ | *In vitro* antiplasmodial activity $IC_{50}$(μM±SD)[a] | | SI[b] | LogP[c] | Resistance factor[d] |
|---|---|---|---|---|---|---|
| | | 3D7 | K1 | | | |
| 4 | H | 0.155±0.017 | 0.137±0.014 | 234.83 | 2.95 | 0.88 |
| 5 | $CH_3$ | 0.250±0.021 | 0.923±0.032 | 428.50 | 3.44 | 3.69 |
| 6 | $CH(CH_3)_2$ | NA | NA | NA | 4.33 | ND |
| 7 | $CH_2CH(CH_3)_2$ | 0.024±0.003 | 0.026±0.007 | 1011.71 | 4.68 | 1.08 |
| 8 | $CHCH_3CH_2CH_3$ | 0.735±0.034 | 1.43±0.045 | 104.59 | 4.75 | 1.36 |
| 9 | $CH_2C_6H_5$ | 0.175±0.039 | 0.304±0.028 | 166.22 | 5.12 | 1.73 |
| 10 | $CH_2$-Indolyl | 0.212±0.026 | 2.08±0.049 | 95.23 | 4.66 | 4.71 |
| 11 | $(CH_2)_2SCH_3$ | 0.386±0.033 | 0.355±0.037 | 92.27 | 3.78 | 0.91 |
| 12 | $CH(CH_3)_2$ | 0.884±0.046 | 0.510±0.036 | 20.50 | 4.32 | 0.57 |
| 13 | $CH_2CH(CH_3)_2$ | 1.69±0.045 | 2.09±0.049 | 34.75 | 4.67 | 1.00 |
| 14 | $CH_2C_6H_5$ | 0.909±0.041 | 1.56±0.048 | 33.89 | 5.11 | 1.10 |
| 15 | $CH_2$-Indolyl | 0.958±0.032 | 0.996±0.038 | 55.17 | 4.65 | 1.03 |

| | | | | | | |
|---|---|---|---|---|---|---|
| CQ |  | 0.005±0.002 | 0.255±0.049 | 8983 | 3.73 | 51 |

[a] $IC_{50}$(μM±SD) : Concentration corresponding to 50% growth inhibition of the parasite; [b] Selectivity index (SI): ($CC_{50}$ for cytotoxicity to vero cells/$IC_{50}$(3D7) for antiplasmodial activity); [c]log P values calculated using ChemBioDraw ultra software; [d] Resistance factor (RI) = $IC_{50}$ (K1)/$IC_{50}$ (3D7); ND: Not done, NA: Not active.

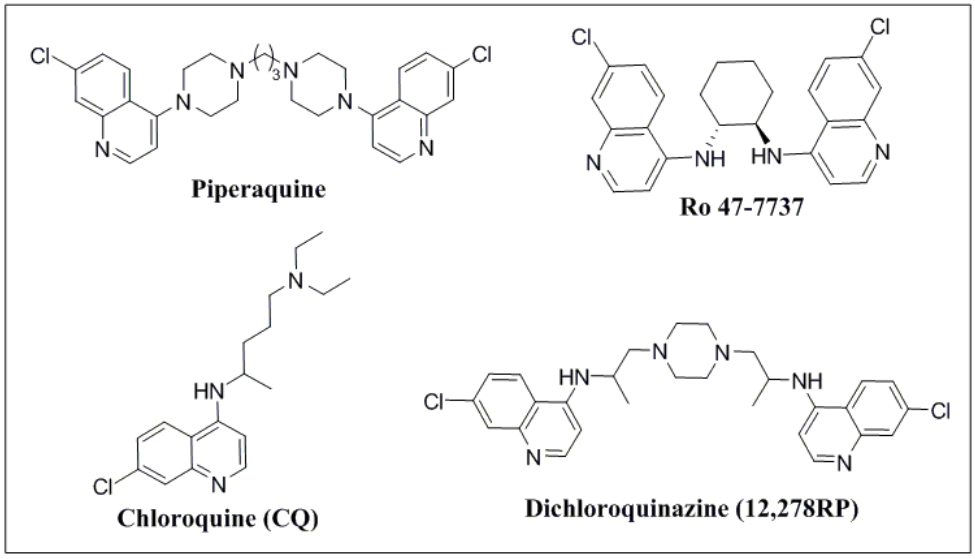



**Figure 1** Structures of some antimalarial agents

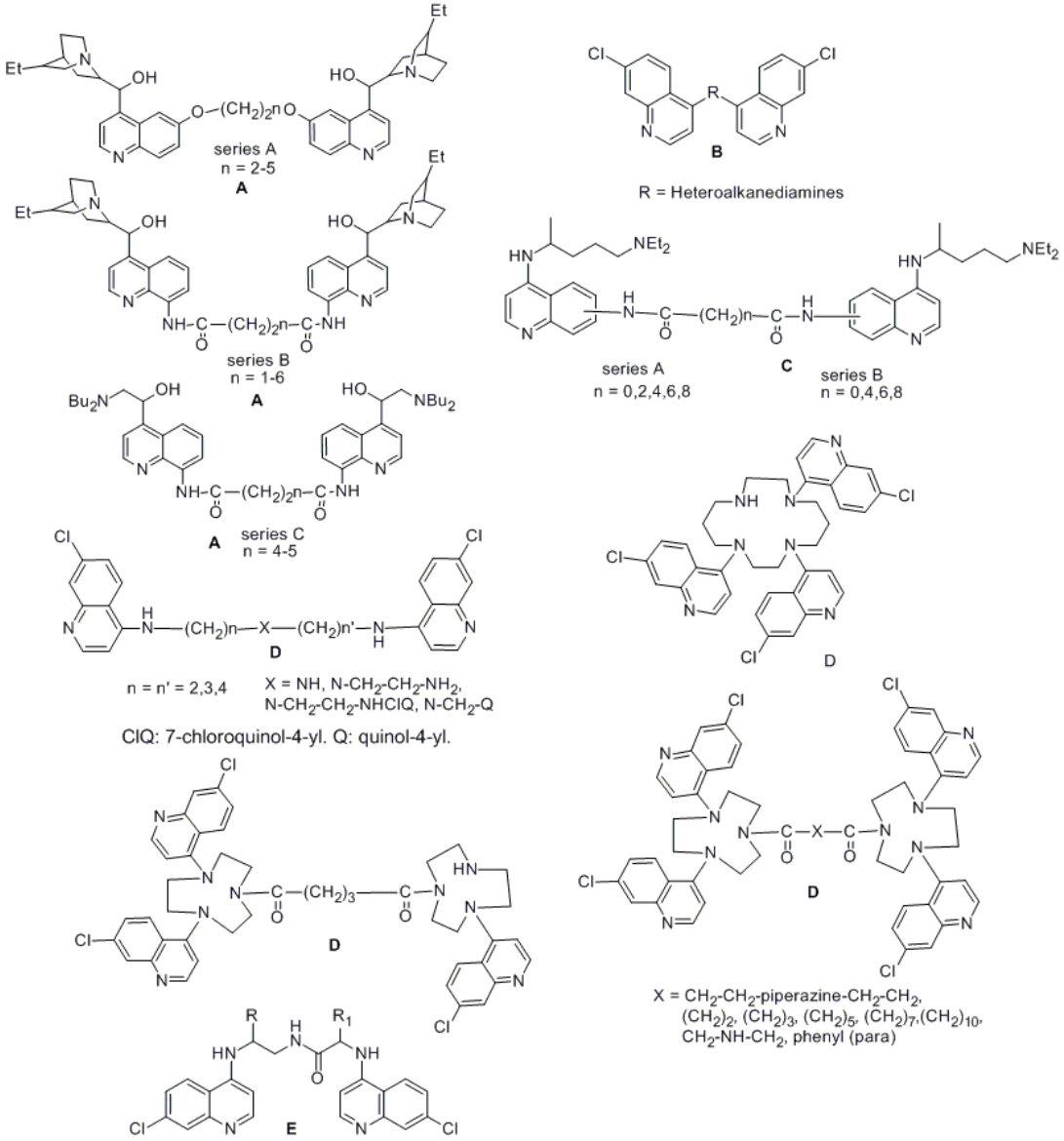



**Figure 2** Structures of some bisquinoline antimalarials.

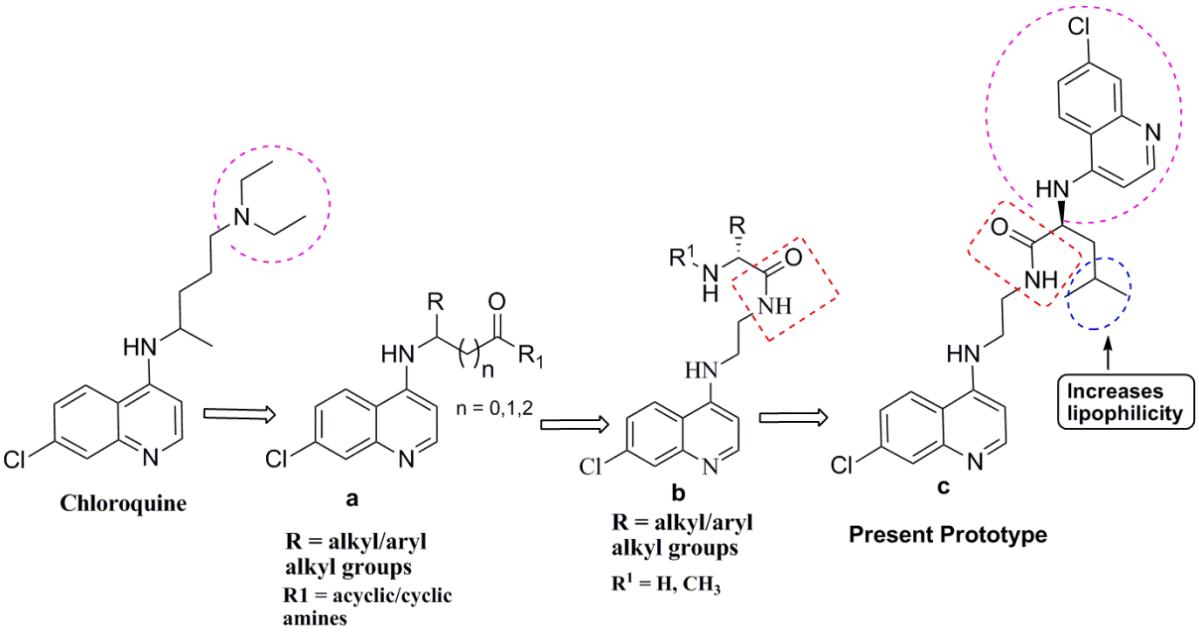



**Figure 3** Some lead molecules of 4-aminoquinoline derived antimalarials developed from this laboratory.

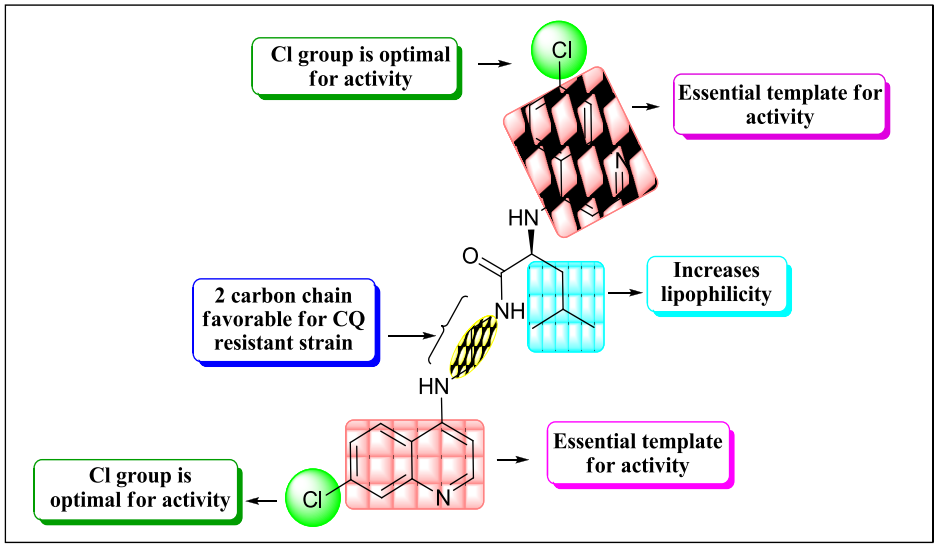
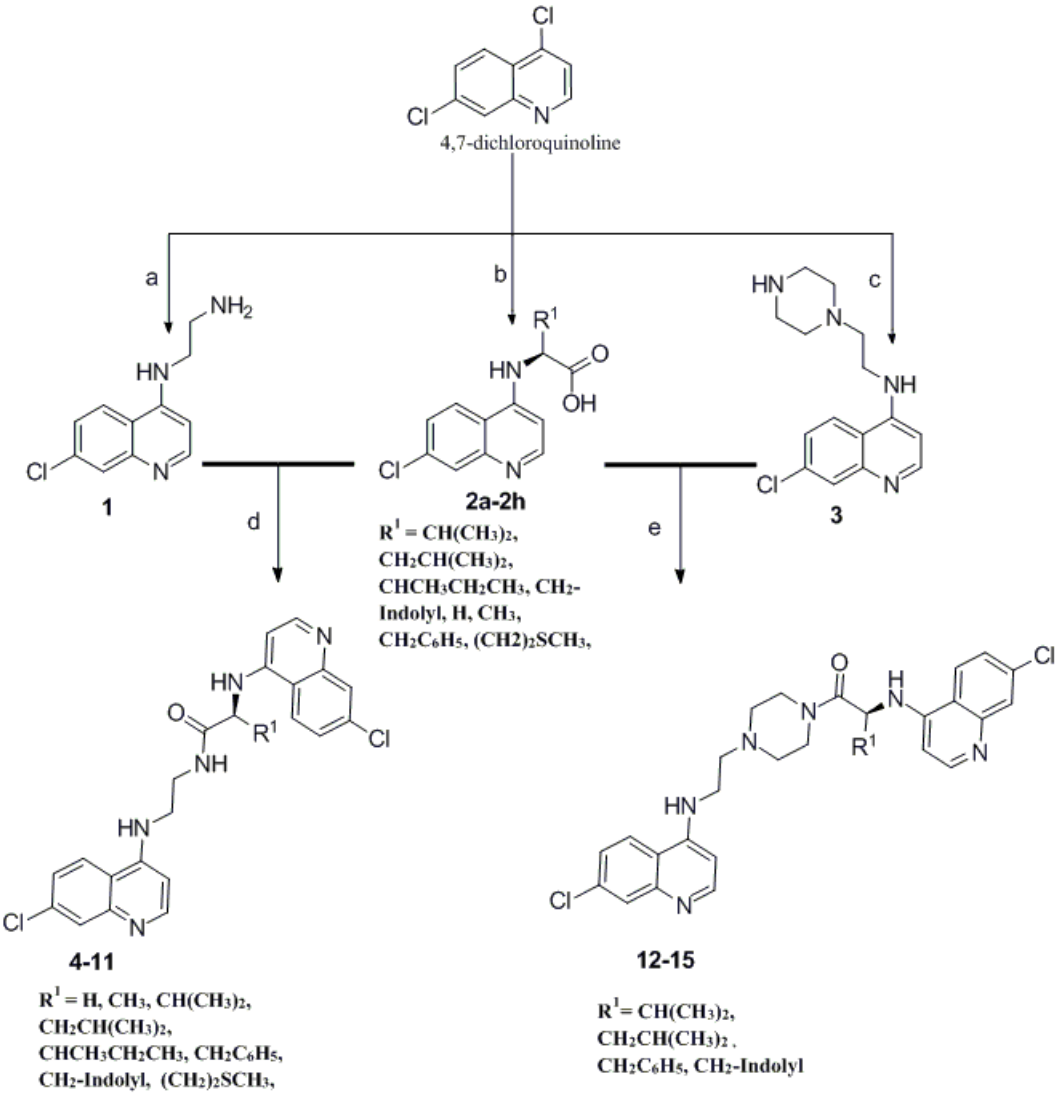



**Figure 4** SAR of the synthesized potent active bisquinoline.




***Scheme 1 Synthesis of bisquinoline antimalarials. Reagents and conditions:*** a) ethylenediamine, neat, 80-130 °C, 8h. b) amino acid, phenol, 160 °C, 1-6 h, 55-68% c) 1-(2-Aminoethyl)piperazine, $CH_3OH$, reflux, 4-8 h, 75% d,e) DCC/HOBt, DMF, 0 °C, 4-6 h, 69-82%.