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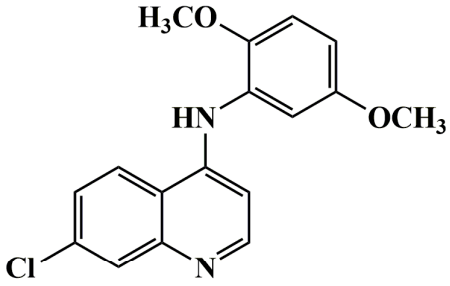
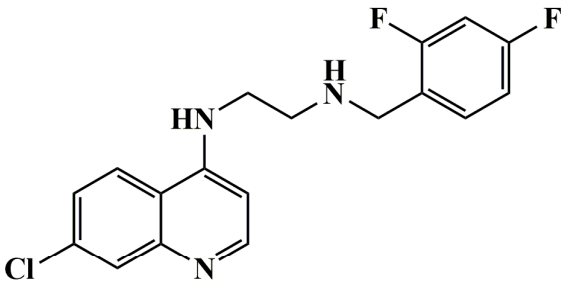


Series I
Compound 1o

IC_{50} = 2.2x10^{-5}, 6.8 x 10^{-5} (g/L)
Mean ED_{50} ± SEa (mg/ Kg B. W.) = 2.231 ± 0.502



Series II
Compound 2j

IC_{50} = 1.1x10^{-5}; 3.4x10^{-5}(g/L)
Mean ED_{50} ± SEa (mg/ Kg B. W.) = 1.623 ± 0.381

4-Aminoquinoline derivatives: Synthesis, *in vitro* and *in vivo* antiplasmodial activity against chloroquine-resistant parasites

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Abstract

Synthetic quinoline derivatives continue to be considered as candidates for new drug discovery if they act against CQ-resistant strains of malaria even after the widespread emergence of resistance to CQ. In this study, we explored the activities of two series of new 4-aminoquinoline derivatives and found them to be effective against *Plasmodium falciparum* under *in vitro* conditions. Further, we selected four most active derivatives 1m, 1o, 2c and 2j and evaluated their antimalarial potential against *Plasmodium berghei in vivo.* These 4-aminoquinolines cured BALB/c mice infected with *P. berghei*. The ED_{50} values were calculated to be 2.062, 2.231, 1.431, 1.623 and 1.18 mg/kg of body weight for each of the compounds 1m, 1o, 2c, 2j and amodiaquine, respectively. Total doses of 500 mg/kg of body weight were well received. The study suggests that these new 4-aminoquinolines should be used for structure activity relationship to find lead molecules for treating multidrug-resistant *P. falciparum* and *P. vivax.*

Keywords: Antimalarial, Amodiaquine, Chloroquine, 4-Aminoquinoline analogues, *Plasmodium falciparum, Plasmodium berghei*

1. Introduction

Malaria mortality rates have come down by a drastic 47% between 2000 and 2013 globally [1]. The improvements are mainly the result of large scale investments and implementation of insecticide treated nets (ITNs), improved diagnostics using rapid diagnostics tests and treatment of malaria using the efficacious antimalarial drug combinations. However, emergence of drug and insecticide-resistance stand ahead as major obstacles, and attempts should be directed at overcoming them as soon as possible, so as to avoid an upsurge in malaria attributed morbidity and mortality.

Combination therapy is undoubtedly the best way to treat malaria and to mitigate the emergence of drug resistance [2]. The World Health Organization (WHO), back in 2001, recommended the use of artemisinin-based combination therapies (ACTs) in endemic areas of Africa and other tropical countries [3]. However, decreased susceptivity of *P. falciparum* to artemisinin pose serious concerns [4] and new drugs and drug combinations are highly needed.

Drug candidates based on the 4-aminoquinoline scaffold continue to be considered as promising candidates for combination therapy [5].Till date, no other drug has been able to match the antimalarial attributes of chloroquine (CQ). However, widespread resistance has rendered it almost useless throughout the world, particularly against *P. falciparum* infections [6]. Amodiaquine (AQ), effective against many chloroquine-resistant strains of *P. falciparum*, was developed by the US Army-sponsored program to develop alternatives to quinine; its use became widespread both prophylactically and therapeutically [7]. Soon after, in the 1980s, its use was terminated because of the occurrence of numerous cases of agranulocytosis, neutropenia and hepatotoxicity in adults taking the drug prophylactically [8]. However, later, its therapeutic use was reiterated and to date, there is no evidence for serious toxicity associated with amodiaquine therapy [9]. AQ toxicity has been explained by the presence of its 4-hydroxyanilino moiety, which is believed to undergo extensive metabolization to its quinoneimine variant (Fig. 1), a feature contrasting to chloroquine [10].

In this article, we describe the synthesis and antimalarial activity of amodiaquine analogues under two series, I and II. These analogues inhibit the formation of toxic metabolites by simple oxidation due to absence of hydroxyl group at position 4 of benzene unlike amodiaquine. Moreover, these are potent against CQ resistant *P. falciparum* parasites. The compounds were tested: (i) as blood

schizonticides against *P. falciparum in vitro*; (ii) against *P. berghei* infections in mice; (iii) for their *in vitro* cytotoxicity; (iv) *in vivo* toxicity; (v) for their binding mode to lactate dehydrogenase and dimeric hematin *in silico*. Apart from an excellent antiplasmodial profile, these analogues are extremely cost-effective to synthesize. On the basis of initial data reported in this paper, selected potent analogues may represent new leads for development of synergistic drug partners in antimalarial combination therapy.

2. Results and Discussion

2.1 Synthesis of 4-aminoquinoline analogues

Most of the clinical antimalarial drugs including chloroquine (CQ) have lost their utility due to the development of drug resistance. A closely related amodiaquine could be applied as an alternate since it retains antimalarial activity against many CQ-resistant parasites [11]. Activity of AQ is linked to a dynamic conformation in which the separation between the quinoline nitrogen and the diethylamino nitrogen is approximately 8.30 A˚, which is very much similar to chloroquine [12]. Research studies reveal that intramolecular hydrogen bond between the hydroxyl and the proton of the charged diethylamino function plays a significant role in the activity of amodiaquine derivative. However, AQ produces a toxic quinoneimine metabolite (Fig. 1) thus; it has been limited since the mid 1980s [13]. According to last WHO's guidelines for treatment of malaria, use of AQ is still recommended either in combination with artemisinin derivatives or with sulfadoxine/pyrimethamine. On careful examination of the structure of amodiaquine, we have found that there is still some scope for modification of basic scaffolds to improve antimalarial activity.

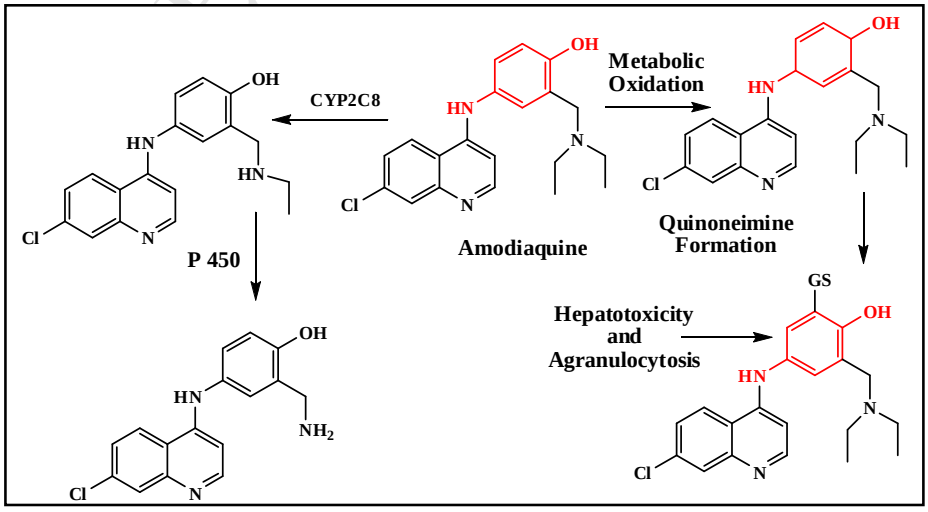


Figure 1. Production of a toxic quinoneimine metabolite by amodiaquine.

In this way newer cost effective compounds with significant antimalarial activity can be designed and synthesized.Till now little systematic study has been carried out using different electron releasing and donating groups on phenyl rings which exhibited the effect of substituents on the activity of the basic scaffold [14]. Keeping these facts in mind and in the continuation of our ongoing projects on malaria drug discovery [15-18] and biophysical analysis of amodiaquine analogues [19], we have synthesized and characterized two different libraries of small 4-aminoquinoline analogues under series I & II as shown in Figure 2.

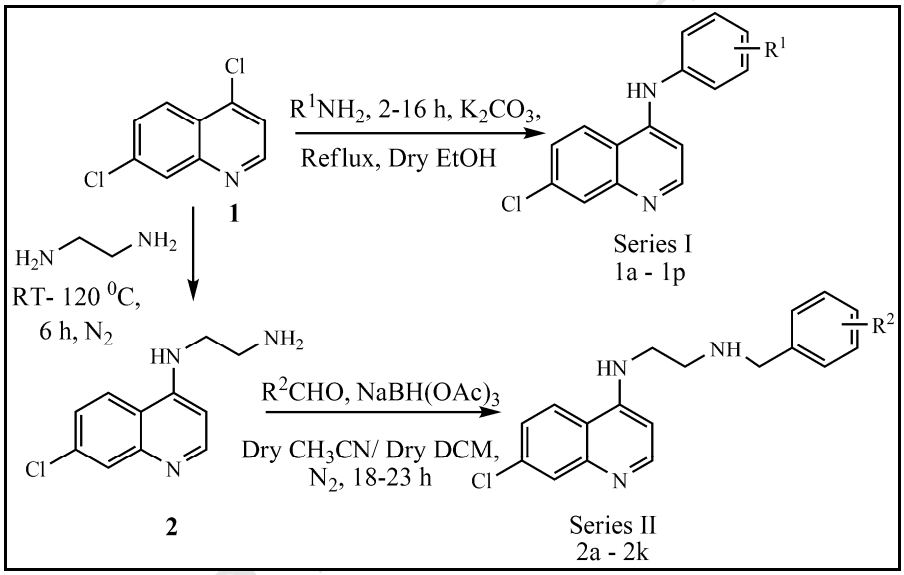


Figure 2. Series I and II showing reagents and conditions used in synthesis of compounds.

Since AQ retains antimalarial activity against many CQ-resistant parasites, several modifications were made to synthesize safer, cost-effective alternative. Other groups have reported the synthesis of fluoro amodiaquine (FAQ) [20]. This analogue cannot form toxic metabolites due to absence of hydroxyl group and retains substantial antimalarial activity versus CQ-resistant parasites [21]. In order to diversify AQ analogues, in this study, we further report the design and synthesis of newer compounds by introducing different functional groups on phenyl ring to improve antimalarial efficacy and lessen toxicity. We assume that these combinations might have significant impact on the parasite life cycle by inhibiting hemozoin formation as well as non-toxic in nature.

We have synthesized 16 analogues (series-I) by direct nucleophilic substitution reaction on 4, 7-dichlroquinoline using various substituted anilines. Further, the introduction of a flexible linear chain between the two amino functions in CQ and the replacement of the terminal diethylamino group by different substituents like alkyl, phenyl with electron donating and electron withdrawing groups were screened and well reported [22]. It is found that 4-aminoquinoline derivatives having 2 or 3 carbon linear chain between two terminal amino groups exhibited promising antimalarial activity (Fig. 3) [23]. These modifications were expected to enhance the lifetime of chloroquine analogues and interesting activity against CQ resistant strains. Keeping the above facts in mind, we have synthesized small library of compounds by reductive amination reaction (series II) in which the length of four carbon linker chain is similar to that of chloroquine except that one carbon is replaced by NH group. Different electron releasing and electron withdrawing groups were placed on o, m or p-positions to get maximum inhibitory activity. All new compounds were well characterized by NMR and mass spectrometry.

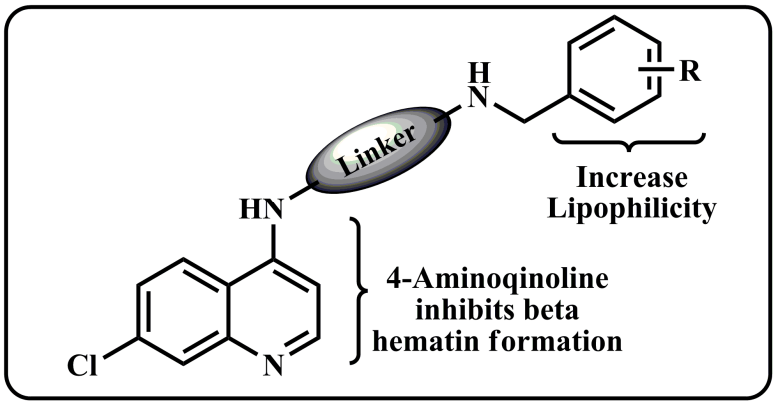


Figure 3. Basis for the synthesis of 4-aminoquinoline derivatives by reductive amination reaction

Under series-I, we have synthesized 16 compounds (Table 1) using different substituted aromatic anilines. Briefly, equimolar amount of 4, 7-dichloroquinoline and desired aniline were reflux in dry ethanol in basic condition (K_2CO_3, 1.2 eq.). Compounds were purified by crystallization in methanol / methanol+acetone or C_2H_5OH as solvent.

All the 11 compounds of series-II (Table 2) were synthesized using N^1-(7-chloroquinolin-4-yl)-ethane-1, 2-diamine (**2**) as starting substrate. In general, commercially available starting material 4, 7-dichloroquinoline was treated with an excess of aliphatic linear chain diaminoalkane via a SNAr type of reaction under usual conditions as reported in the literature to afford substituted 4-

aminoquinolines with free terminal amino groups in good yield [24]. This intermediate on reaction with suitable aldehydes yielded desired product in minor to low yield [25].

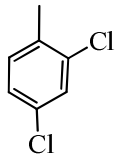
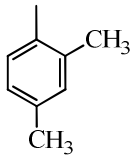
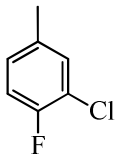
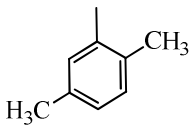
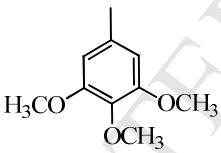
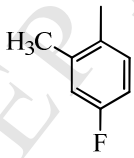
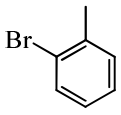
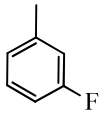
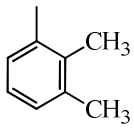
All the compounds synthesized by the above given procedure were purified either by crystallization or by column chromatography and characterized by different spectroscopic techniques *viz.* IR, ^{1}H & ^{13}C-NMR and MASS. Figure 2 summarizes the synthesis strategy behind series-I and II.

2.2 Efficacies of 4-aminoquinoline analogues against *P. falciparumin vitro*

Two newer series of AQ analogues, series-I (**1a-p)** and series-II (**2a-k)** were evaluated *in vitro* against the erythrocytic stages of *P. falciparum* (FCR3 strain, a chloroquine, pyrimethamine and cycloguanil resistant strain). Results in Table 1 show that most of the new compounds of series-I exhibited very promising activity ranges, depending on the functional group on phenyl ring. The most potent compounds were 1m (IC_{50} 95% Confidence intervals = [$2.9x10^{-5}$; $9.3x10^{-5}$]g/L), which contains fluoro group at para position and 1o (IC_{50} 95% Confidence intervals = [$2.2x10^{-5}$; $6.8x10^{-5}$]g/L), with 2, 5-dimethoxy groups on phenyl ring. However, the compound 1e having three methoxy groups at 3, 4, 5-positions of the phenyl ring showed significant loss of activity as compared to the compound 1o. This could be attributed to steric hindrance caused by the presence of three methoxy groups. Further, compounds 1h, 1i, 1j, 1k, 1l and 1p exhibit moderate activity, while compound 1c shows least antimalarial activity in comparison to the most active compounds in series I.

In series-II, compound 2c (IC_{50} 95% Confidence intervals = [$2.1x10^{-5}$; $5.6x10^{-5}$]g/L) and 2j (IC_{50} 95% Confidence intervals = [$1.1x10^{-5}$; $4x10^{-5}$]g/L) were most active. This result signifies that fluoro and bromo at para position in phenyl ring impart significant antimalarial activity. The electron releasing groups such as methoxy and methyl at para position on phenyl ring (compounds 2a and 2i, respectively) also enhance antimalarial activity while chloro group (compound 2b) has lesser effect on antimalarial activity. Further, compounds 2d, 2e, 2g and 2h display moderate while compound 2f shows least antimalarial efficacy with respect to the most active compounds. Interestingly, 3, 5-dimethoxy group enhance antimalarial activity in series I while it has lesser effect in series II. The reason behind such varying behavior of compounds with similar nature of functional groups is still elusive. This study clearly demonstrates that there is need of systematic SAR study to establish meaningful correlation among various functional groups. Work in this direction is under progress.

Table 1. List of compounds synthesized using series-I, along with their antiplasmodial activities against *P. falciparum* FCR3 strain and cytotoxicities against Huh-7 cells.

Compound Code	R^1	IC_{50} 95% Confidence intervals (g/L)	Mean CC_{50}± SE[a] (µg/mL)
1a		[$7.6x10^{-4}$; $3.9x10^{-3}$]	>100µg/mL
1b		[$4.0x10^{-4}$; $1.3x10^{-3}$]	>100µg/mL
1c		[$1.9x10^{-3}$; $5.5x10^{-3}$]	>100µg/mL
1d		[$4.6x10^{-4}$; $1.8x10^{-3}$]	>100µg/mL
1e		[$6.4x10^{-4}$; $3.2x10^{-3}$]	>100µg/mL
1f		[$7.6x10^{-5}$; $4.3x10^{-4}$]	>100µg/mL
1g		[$1.7x10^{-4}$; $6.2x10^{-4}$]	>100µg/mL
1h		[$9.8x10^{-5}$; $2.7x10^{-4}$]	>100µg/mL
1i		[$3.6x10^{-5}$; $1.3x10^{-4}$]	>100µg/mL

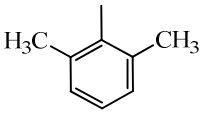
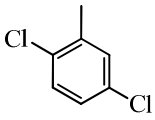
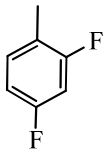
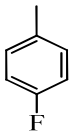
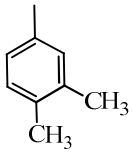
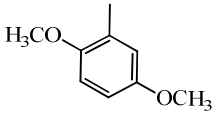
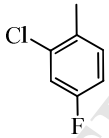
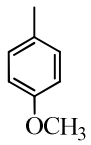
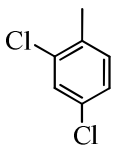
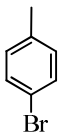
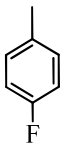
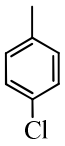
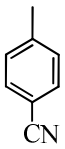
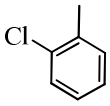
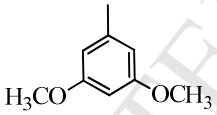
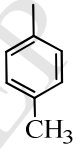
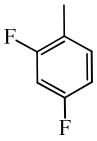
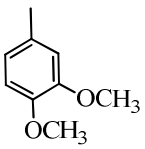
1j		[4.6x10^{-5}; 1.5x10^{-4}]	>100µg/mL
1k		[5.6x10^{-5}; 1.9x10^{-4}]	>100µg/mL
1l		[4.5x10^{-5}; 1.8x10^{-4}]	>100µg/mL
1m		[2.9x10^{-5}; 9.3x10^{-5}]	>100µg/mL
1n		[1.6x10^{-4}; 5.0x10^{-4}]	>100µg/mL
1o		[2.2x10^{-5}; 6.8x10^{-5}]	>100µg/mL
1p		[6.5x10^{-5}; 2.1x10^{-4}]	>100µg/mL

Table 2. List of compounds synthesized using series-II, along with their antiplasmodial activities against *P. falciparum* FCR3 strain and cytotoxicities against Huh-7 cells.

Compound Code	**R^2**	**IC$_{50}$ 95% confidence interval(g/L)**	**Mean CC$_{50}$± SE[a](µg/mL)**
2a		[1.3x10^{-5}; 3.4x10^{-5}]	>100µg/mL
2b		[1.9x10^{-3}; 3.0x10^{-3}]	>100µg/mL

2c		[$2.1x10^{-5}$; $5.6x10^{-5}$]	>100µg/mL
2d		[$3.0x10^{-5}$; $1.2x10^{-4}$]	>100µg/mL
2e		[$2.8x10^{-5}$; $1.2x10^{-4}$]	>100µg/mL
2f		[$1.8x10^{-3}$; $1.1x10^{-2}$]	39.58µg/mL
2g		[$5.0x10^{-5}$; $1.9x10^{-4}$]	>100µg/mL
2h		[$7.2x10^{-5}$; $4.4x10^{-4}$]	>100µg/mL
2i		[$1.8x10^{-5}$; $6.6x10^{-5}$]	>100µg/mL
2j		[$1.1x10^{-5}$; $3.4x10^{-5}$]	>100µg/mL
2k		[$1.4x10^{-5}$; $3.5x10^{-5}$]	>100µg/mL

2.3 *In vitro* cytotoxicity assay

To substantiate the therapeutic value of compounds in series-I and series-II, toxicity on mammalian cells was assessed against Huh7 cells; a hepatocyte derived cellular carcinoma cell line (Tables 1 and 2). However, only compound 2f, containing nitrile group at para position of phenyl ring appeared to be associated with some toxicity (CC_{50} value of 39.58μg/mL). All the other compounds are devoid of any considerable cytotoxicity at the highest test concentration (100μg/mL). Thus, these results further support the significance of this study.

2.4 *In vivo* activities of selected derivatives against rodent malaria

We chose 4 compounds that displayed the most potent *in vitro* activity (compounds **1m** and **1o** from series I; compounds **2c** and **2j** from series II) for evaluation *in vivo*, against the rodent malaria parasite *P. berghei* (erythrocytic stage of ANKA, a CQ resistant strain). After conducting Peters' four-day suppressive test, it was observed that there was a reduction in the levels of parasitemia in all the test groups, as well as that of the standard drug (AQ) group. However, the reverse was the case for the negative control group, as there was a marked increase in parasitemia level on day 4. The *in vivo* antimalarial activity of the various test compounds is presented in Figure 4.

Results were significant as analyzed by ANOVA ($P < 0.05$). The ED_{50} values were calculated to be 2.062, 2.231, 1.431, 1.623 and 1.18mg/kg BW for each of the compounds **1m**, **1o**, **2c**, **2j** and amodiaquine respectively, as indicated in Table 3. The mean survival time (MST) values of the animals in test groups were determined, demonstrating a dose-dependent increase in the number of days the mice survived in various groups survived post-four-day treatment. MST values of the treated groups were significantly higher than that of control and were comparable to that of the standard drug (Fig. 5). All the results were significant ($P < 0.005$) as analyzed by Log-rank (Mantel-Cox) test. No significant side-effects such as weight loss were observed in compound treated groups.

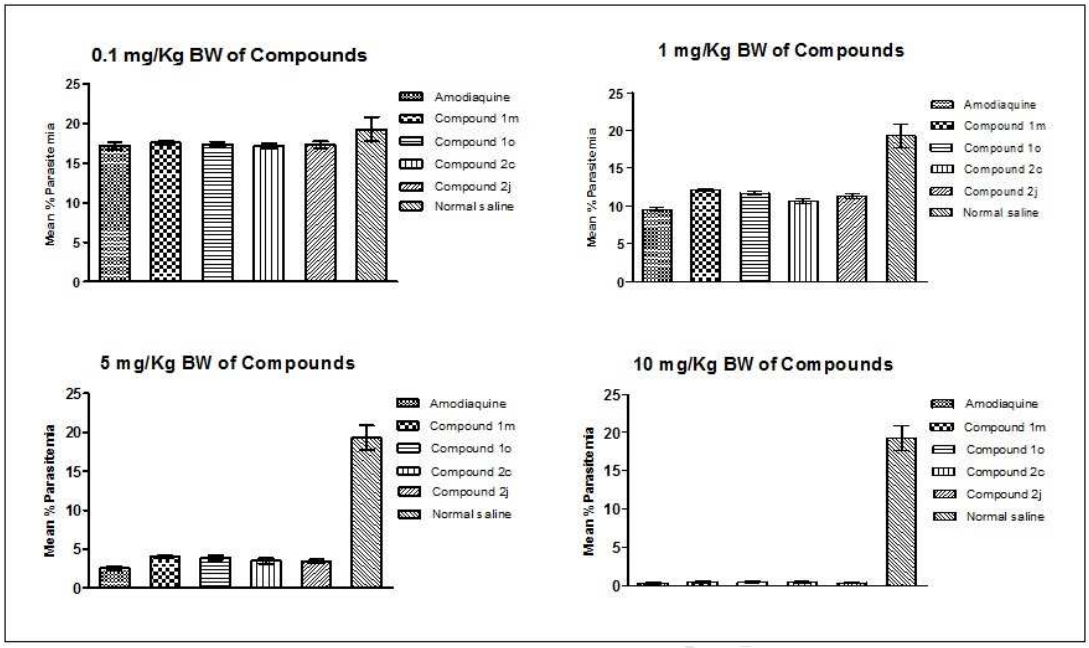


Figure 4. Effects of various compounds and amodiaquine on established *P. berghei* infections in mice. The experimental hosts were infected on day 0 and treated intraperitoneally with normal saline; compounds 1m, 1o, 2c, 2j and amodiaquine at 0.1, 1.0, 5, or 10 $mg \cdot kg^{-1}BW \cdot day^{-1}$on days 0 to 3, as described by Ryley and Peters. Data expressed as Mean ± SD of five mice per condition.

Table 3. Antiplasmodial activity of amodiaquine and derivatives against *P. berghei* ANKA strain.

Compound Code	**Mean ED_{50} ± SE[a] (mg/ Kg B. W.)**	**Percentage Inhibition (on day 4)**			
		0.1 mg/ Kg	1 mg/ Kg	5 mg/ Kg	10 mg/ Kg
1m	2.062 ± 0.433	21.63	32.027	54.19	79.13
1o	2.231 ± 0.502	11.985	21.68	58.073	93.363
2c	1.431 ± 0.353	25.653	45.253	58.79	82.70
2j	1.623 ± 0.381	8.413	36.73	67.597	97.357
Amodiaquine	1.187 ± 0.256	28.023	48.24	67.137	94.07

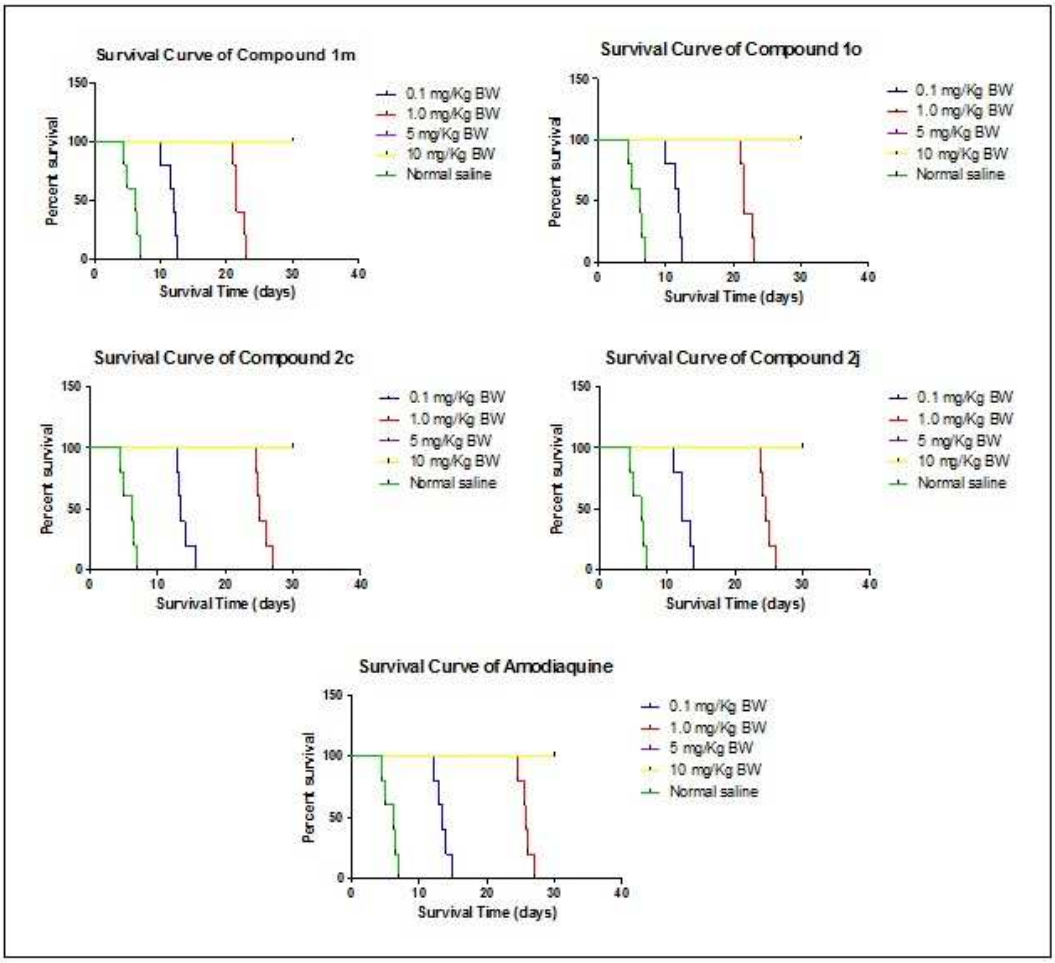


Figure 5. Kaplan Meier survival analysis curves of BALB/c mice, administered drugs once daily ip for four consecutive days, as described by Ryley and Peters (5 mice per group). Results between test and control were significant by $P < 0.005$ as analyzed by Log-rank test.

* Activity in 5 mg/Kg BW treated groups is being superimposed by the activity in 10 mg/Kg BW treated groups.

2.5 Favourable safety profiles in test animals

In the LD_{50}test for determining the therapeutic indices of compounds, BALB/c mice died at1000mg/kg BW of all the compounds and could tolerate 500mg/kg BW. However, at 800mg/kg BW, half the population of mice died. Therapeutic indices were determined as 387.973, 358.584, 559.05and 492.914 for compounds **1m**, **1o**, **2c** and **2j**, respectively.

2.6 *In silico* studies

2.6.1 Inhibition of hemozoin formation

Whether anti *P. falciparum* activity of the selected derivatives involves inhibition of hemozoin formation was evaluated by docking them with dimeric hematin. Docking studies with compounds **1m**, **1o**, **2c** and **2j** showed that the H-bond energies between the antimalarial compounds and the dimeric hematin (Table 4) were approximately -6.0 kcal mol^{-1}, ascertaining that hydrophobic interactions were the main contributors for the binding. Thus, all four AQ derivatives, similar to CQ [26], were able to interact with dimeric hematin to form a complex, which in turn is responsible for the hindrance caused to heme polymerization. The best conformations obtained for the complexes showed that the aromatic rings of all the compounds were parallel to the hematin ferriprotoporphyrin group (Fig. 6).

Table 4. Docking energies of the 4-aminoquinoline analogues with dimeric hematin.

Compound	**H Bond Energy (Kcal/ mol^{-1})**
1m	-6.01
1o	-5.98
2c	-5.81
2j	-5.13

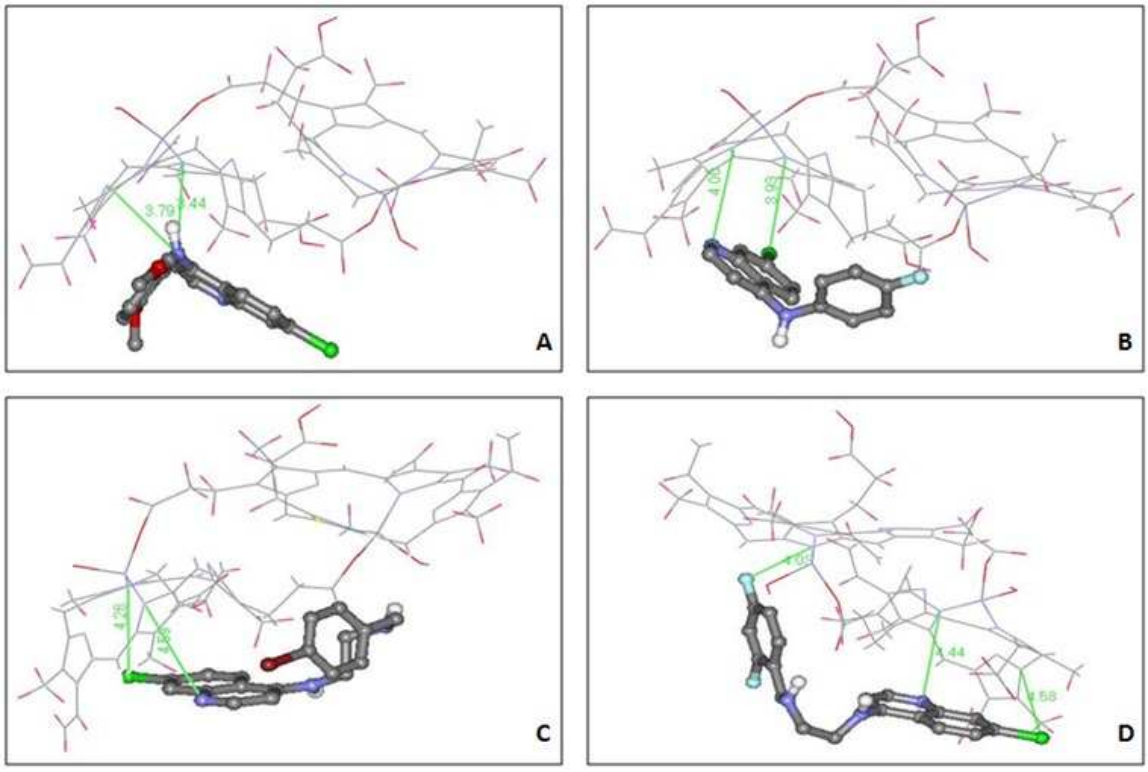


Figure 6. Compounds docked in dimeric hematin, illustrating the formation of complexes to inhibit hemozoin polymerization. (A) 1m, (B) 1o, (C) 2c, (D) 2j.

2.6.2 Binding to lactate dehydrogenase (LDH) contributes to the antimalarial action

Table 5 presents the H-bond energy of the residues involved in H-bonds with compounds **1m**, **1o**, **2c** and **2j**, docked to PfLDH. Figure 6 shows that these compounds presented the most stable energy conformations in the binding site of NADH. This suggests that they are NADH competitors. The H-bond energies between the amodiaquine derivatives and the enzyme were considerably high, as compared with that of NADH H-bond energy (-35.3 kcal mol^{-1}) [26]. Furthermore, 2j presented the lowest energy, which was quite large when compared to NADH. These results imply that all the test compounds are weak inhibitors of PfLDH, as previously reported for CQ [27].

Although PfLDH is not the principal target of CQ, experimental data and modeling studies have explored its weak binding to CQ [28]. The existence of this shared target-binding site suggests that PfLDH has an inevitable role to play in drug effectiveness. The docking data (Table 5) suggest that the compounds present the most stable energetic conformations at the binding site of NADH inside PfLDH. The H-bond energy, residues involved in the H-bonds with NADH, residues contributing to vanderwaals and other weak interactions and the compounds bound to PfLDH (Fig. 7) suggest that these compounds are NADH competitors. Overall, these findings stand in strong support of these compounds acting as weak inhibitors of PfLDH, as has been observed in the case of chloroquine [29, 30].

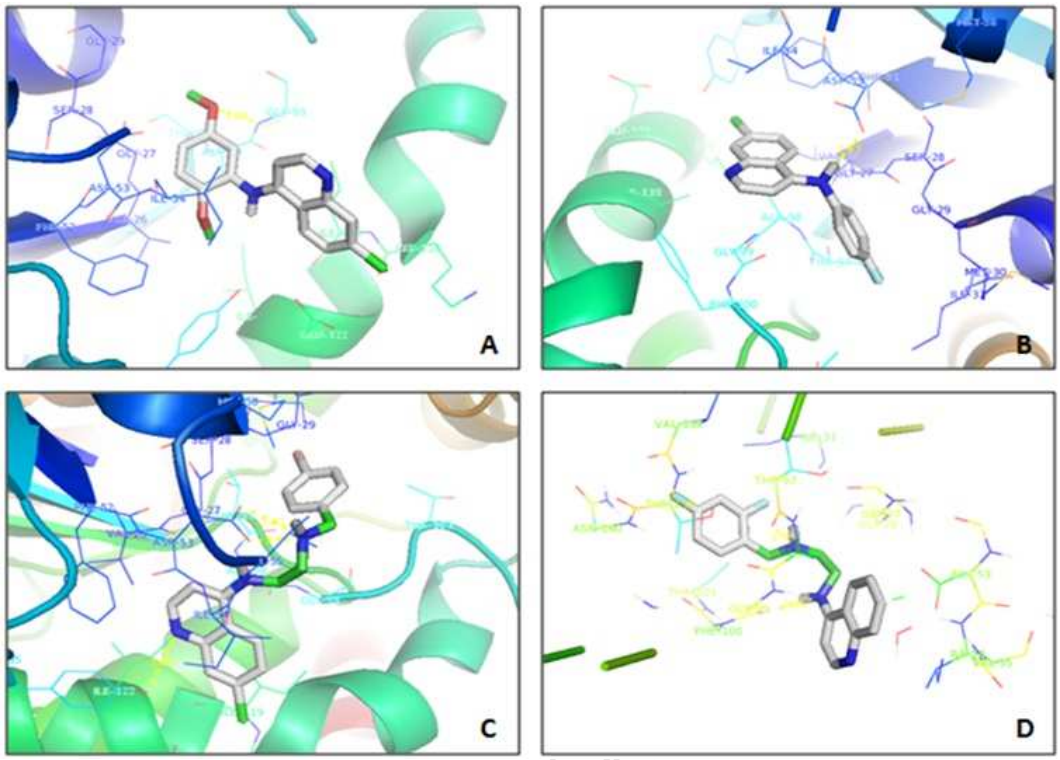


Figure 7. Best conformations of the amodiaquine derivatives in the binding pocket of NADH as generated by the Autodock software. (A) 1m, (B) 1o, (C) 2c, (D) 2j.

Table 5. Docking results of the 4-aminoquinoline analogues in the active site of PfLDH.

Compound	**H Bond Energy (Kcal/ mol^{-1})**	**Residues (H Bond Interactions)**	**Residues (Vander waals/ other weak Interactions)**
1m	-6.21	Gly99	Asp53, Ile54, Tyr85, Ala98, Lys118, Ile119, Glu122, Ile123
1o	-6.5	Asp53	Gly27, Ser28, Gly29, Met30, Ile31, Val36, Asp53, Ile54, Thr97, Ala98, Gly99, Phe100, Thr101,Ile119,Glu122
2c	-7.85	Val55, Tyr85	Gly99, Glu122, Lys118, Ile123, Gly32, Thr101, Phe52.
2j	-5.13	Gly99, Thr97	Gly27, Gly29,Ile31, Ile54,Thr97, Ala98, Gly99, Phe100, Thr101, Val138, Thr139, Asn140

3. Conclusion

We have successfully designed, synthesized and characterized two series of new amodiaquine derivatives utilizing readily available and inexpensive chemicals. The present study has led to the identification of four antiplasmodial lead compounds: 1m and 1o from series-I; 2c and 2j from series-II. These lead compounds display significant activity and low or no toxicity, both *in vitro* and *in vivo* and could be converted to more potent, synergistic antimalarial drug partners to be employed in the combination therapy approach. The preliminary screening of antimalarial activity should encourage further detail studies aimed at optimizing their antimalarial activities.

4. Experimental procedure

4.1 General methods

Various chemicals and solvents used in this study were purchased from E. Merk (India) and Sigma-Aldrich chemicals. The progress of reactions was monitored by silica gel thin layer chromatography (TLC) plates and visualized under UV. Melting points were determined by using open capillary method and Buchi Melting Point M 560 melting apparatus and are uncorrected. ^{1}H-NMR and ^{13}C-NMR spectral data were recorded on BruckerAvance spectrometer at 300 MHz and Jeol JNM spectrometer at 400 MHz, respectively in $CDCl_3$ or CD_3OD or DMSO-d_6, using TMS as an internal standard. The chemical shift values were recorded on δ scale and the coupling constants (*J*) in hertz. The following abbreviations were used in reporting spectra: s = singlet, d = doublet, t = triplet, q = quartet, m = multiple. IR spectra were obtained on a Perkin Elmer Fourier-transform infrared (FT-IR) Spectrophotometer (Spectrum 2000) in potassium bromide disk. ESI-MS spectra were obtained on a Waters micromass LCT Mass spectrometer. Elemental analysis was done on Elementar GmbH VarioElanalyser. MS and MS/MS data were also recorded automatically on the MALDI-TOF/TOF instrument. Spectra were recorded in the reflectron mode using an Ultraflex III Tof/Tof mass spectrometer (Bruker Daltonics) equipped with a 384-sample scout source. The ion acceleration voltage after pulsed extraction was 29000 V. To record the spectra, compounds were mixed with an acidic solid matrix such as α-cyano-4-hydroxy cinnamic acid (CHCA) matrix 10 mg/ml, which provides high sensitivity and negligible matrix adduction during the laser absorption and subjected to laser radiation. The matrix was made in 70% acetonitrile and 0.03% TFA.

4.2 General procedure for the synthesis of N^1-(7-chloro-quinolin-4-yl)-ethane-1, 2-diamine (2)

A mixture of 4,7-dichloroquinoline (1, 5.0 g, 0.025 mol) and 1,2- ethylene diamine (5.8 g, 0.125 mol) was heated slowly from RT to 120 °C and the reaction mixture was stirred at this temperature for 6 h (Series II). After that the reaction mixture was cooled down to room temperature and ice cold water was added to it. The solid thus obtained was filtered and washed with excess water. The crude product was crystallized by using ethanol and the data corresponds to that reported in the literature. [31]

4.3 General procedure for synthesis of various 7-chloroquinolin-4-yl-phenylamine (1a-1p)

Compounds were synthesized using 4, 7-dichloroquinoline and suitable substituted anilines. Briefly, equimolar quantities of anilines (1 mmol) and 4, 7-dichloroquinoline (1 mmol) were refluxed in dry ethanol in basic medium (1.2 mmol K_2CO_3) for 2-16 h depending upon the various substituents. Progress of reaction was monitored by thin layer chromatography. After completion of reaction, reaction mixture was neutralized by 1N NaOH solution and extracted with 3 x 50 mL of $CHCl_3$, washed with $NaHCO_3$ and brine. The organic layer was dried over anhydrous Na_2SO_4. The solvent was again evaporated under reduced pressure. The ligands were purified by successive recrystallization. [32]

Characterization data

Compound 1a: Yield=79%; mp=274-278 °C; Ms: m/z 323 $[M + 1]^+$; ^{1}H-NMR (300 MHz, $CDCl_3$, ppm): δ 8.52 (d, 1H, J=9.0 Hz, Ar-H), δ 8.34 (d, 1H, J=6.9 Hz, Ar-H), δ 8.02 (s, 1H, Ar-H), δ 7.35-7.47 (m, 4H, J=6.9 Hz, Ar-H + NH), δ 7.70 (d, 1H, J=4.8Hz, Ar-H), δ 7.47 (s, 1H, NH), δ 7.41 (s, 1H, Ar-H), δ 7.35 (1H, Ar-H), δ 6.91 (d, 1H, J= 4.9 Hz, Ar-H); ^{13}C-NMR (300 MHz, $CDCl_3$, ppm): δ 168.54, 159.40, 150.91, 143.53, 138.57, 130.87, 129.59, 128.30, 126.76, 122.83, 122.79, 119.65, 118.91, 114.75, 114.53; Anal. Calcd.for $C_{15}H_9Cl_3N_2$: C, 54.98, H, 2.78; N, 5.15. Found: C, 55.20; H, 2.52; N, 6.68; IR (KBr, cm^{-1}): 3448.63, 2593.15, 1608.33, 1369.54, 1207.25, 1093.78.

Compound 1b: Yield=62%; mp=308-312 °C; Ms: m/z 283 $[M + 1]^+$; ^{1}H-NMR (300 MHz, $CDCl_3$ ppm): δ 3.15 (s, 3H, CH_3), δ 3.18 (s, 3H, CH_3), δ 6.33 (d, 1H, J=5.1 Hz, Ar-H), δ 6.47 (s, 1H, NH), δ 8.45 (d, 1H, J=5.1 Hz, Ar-H), δ 8.01 (d, 1H, J= 1.5Hz, Ar-H), δ 7.14 (d, 1H, J=7.5 Hz, Ar-H), ,δ 7.86 (d, 1H, J=9.0Hz, Ar-H), δ 7.45 (d, 1H$_3$, *J*=3.0 Hz), δ 7.14 (2H, Ar-H), δ 7.07 (d, 1H, J=7.8 Hz, Ar-H) ^{13}C-NMR (300 MHz, $CDCl_3$ ppm): δ 151.97, 149.51, 148.83, 136.65, 135.11, 134.52, 134.12, 132.16, 128.96, 127.93, 126.29, 125.78, 121.09, 117.42, 101.69, 20.99, 17.68; Anal.

Calcd.for $C_{17}H_{15}ClN_2$: C, 72.35, H, 5.22; N, 9.93. Found: C, 71.68, H, 6.20; N, 10.23; IR (KBr, cm^{-1}): 3184.15, 2870.07, 1653.93, 1450.62, 1329.59.

Compound 1c: Yield=81%; mp= 298-300°C; Ms: m/z 307 $[M + 1]^+$; ^{1}H-NMR (300 MHz, DMSO-d_6, ppm): δ 8.85 (d, H, J=8.4 Hz, Ar-H), δ 8.19 (s, 1 H, Ar-H), δ 8.56 (d, 1H, J=5.1 Hz, Ar-H), δ 7.79 (s, 1H, Ar-H), δ 6.83 (d, 1H, J=6.6 Hz, Ar-H), δ 7.54-7.64 (m, 2H, Ar-H), δ 5.89 (s, br, 1H, NH); ^{13}C-NMR (300 MHz, DMSO-d_6, ppm): δ 155.00, 143.52, 138.99, 138.45, 134.34, 127.85, 127.45, 126.60, 126.5, 126.35, 120.61, 120.36, 119.17, 117.92, 100.64; Anal. Calcd.for $C_{15}H_9Cl_2FN_2$: C, 58.95; H, 2.66; N, 9.19. Found: C, 60.10; H, 2.32; N, 7.89; IR (KBr, cm^{-1}): 3115.37, 3052.15, 2879.07, 2585.09, 1420.19, 1231.56.

Compound 1d: Yield=58%; mp>310-313 °C; Ms: m/z 283 $[M + 1]^+$; ^{1}H-NMR (300 MHz, CD_3OD, ppm): δ 2.35 (s, 6H, CH_3), δ 8.85 (d, 1H, J=9.03 Hz, Ar-H), δ 8.31 (s, 1H, Ar-H), δ 7.67 (d, 1H, J=8.5 Hz, Ar-H), δ 7.50 (d, 1H, J=7.7 Hz, Ar-H), δ 7.12 (d, 1H, J=3.1 Hz, Ar-H), δ 6.20 (s, 1H, NH), δ 6.5 (d, 1H, J=4.9 Hz, Ar-H), δ 6.7 (d, 1H, J=5.4 Hz, Ar-H); ^{13}C-NMR (300 MHz, CD_3OD, ppm): δ 153.02, 151.91, 149.82, 134.60, 133.10, 128.43, 124.56, 123.99, 127.32, 115.21, 110.48, 128.22, 117.24, 115.12, 103.01, 20.20, 12.44; Anal. Calcd.for $C_{17}H_{15}ClN_2$: C, 72.21; H, 5.35; N, 9.91. Found: C, 70.90; H, 5.63; N, 10.10; IR (KBr, cm^{-1}): 3180.15, 3004.73, 1540.87, 1231.56, 1056.85.

Compound 1e: Yield=85%; mp=313-317 °C; Ms: m/z 345 $[M + 1]^+$; ^{1}H-NMR (400 MHz, DMSO-d_6, ppm): δ 3.70 (s, 3H, OCH_3), δ 3.78 (s, 6H, OCH_3), δ 6.06 (s, br, 1H, NH), δ 6.81 (s, 2H, Ar-H), δ 6.86 (d, 1H, J=5.1Hz), δ 7.86 (d, 1H, J=6.7 Hz, Ar-H), δ 8.14 (s, 1H, Ar-H), δ 8.48 (d, 1H, J = 5.1 Hz, Ar-H), δ 8.78 (d, 1H, J = 6.9 Hz, Ar-H). ^{13}C-NMR (400 MHz, DMSO-d_6, ppm): δ 155.10, 153.68, 143.37, 139.02, 138.38, 136.63, 132.56, 127.38, 125.94, 119.25, 115.75, 103.21, 100.87, 60.19, 56.14; Anal. Calcd.for $C_{18}H_{17}ClN_2O_3$: C, 62.28; H, 4.12; N, 8.70. Found: C, 63.24, 3.70, 9.21; IR (KBr, cm^{-1}): 3006.19, 2901.43, 1607.65, 1560.24, 1330.02, 1242.00.

Compound 1f: Yield=55%; mp=238-240 °C; Ms: m/z 286 $[M + 1]^+$; ^{1}H-NMR (300 MHz, DMSO-d_6, ppm): δ 2.55 (s, 3H, CH_3), δ 8.85 (d, 1H, J=8.01 Hz, Ar-H), δ 8.31 (s, 1H, Ar-H), δ 7.67 (d, 1H, *J*=5.3 Hz, Ar-H), δ 7.50 (d, 1H, J=7.2 Hz, Ar-H), δ 6.55 (d, 2H, J =4.7, Ar-H), δ 6.44 (s, 1H, NH), δ 6.40 (d, 1H, J=7.1 Hz, Ar-H); ^{13}C-NMR (300 MHz, DMSO-d_6, ppm): δ 153.11, 151.59, 149.81, 134.65, 133.01, 128.13, 126.77, 123.92, 127.30, 115.22, 110.74, 128.29, 117.23, 115.12, 103.18,

23.53; Anal. Calcd.for $C_{16}H_{12}ClFN_2$: C, 67.02; H, 9.77; N, 8.70. Found: C, 66.80; H, 10.14; N, 9.23; IR (KBr, cm^{-1}): 3279.75, 2809.57, 2036.49, 1615.49, 1337.05, 1239.84, 1164.43.

Compound 1g: Yield=67%; mp>298-302 °C; Ms: m/z 333 $[M + 1]^+$; ^{1}H-NMR (300 MHz, DMSO-d_6, ppm): δ 8.85 (d, 1H,J=9.03 Hz, Ar-H), δ 8.31 (s, 1H, Ar-H), δ 7.67 (d, 1H, J= 4.3 Hz, Ar-H), δ 7.50 (d, 1H, J=5.7 Hz, Ar-H), δ 7.74-7.65 (m, 4H, J = 25.2 Hz, Ar-H), δ 6.41 (s, 1H, NH); ^{13}C-NMR (300 MHz, DMSO-d_6, ppm): δ 158.12, 151.45, 149.81, 134.62, 133.02, 128.13, 126.75, 123.93, 127.33, 115.20, 110.47, 128.27, 117.23, 115.24, 103.13; Anal. Calcd.for $C_{15}H_{10}BrClN_2$: C, 54.00; H, 3.02; N, 23.95. Found: C, 55.12, 2.86; N, 22.41; IR (KBr, cm^{-1}): 3185.38, 2880.13, 2845.73, 1660.02, 1551.02, 1329.89, 1291.91.

Compound 1h: Yield=87%; mp=317-319 °C; Ms: m/z 273 $[M + 1]^+$; ^{1}H-NMR (400 MHz, DMSO-d_6, ppm): δ 8.91 (d, 1H, J=6.9 Hz, Ar-H), δ 8.54 (d, 1H, J = 5.1Hz, Ar-H), δ 8.20 (d, 1H, J=7.3 Hz, Ar-H), δ 7.52 (d, 1H, J=5.7 Hz, Ar-H), δ 7.40 (t, 1H, J=18.3 Hz, Ar-H), δ 6.90 (d, 1H, J=5.1 Hz, Ar-H), δ 6.87 (s, br, 1H, NH); ^{13}C-NMR (400 MHz, DMSO-d_6, ppm): δ161.23, 154.48, 143.60, 139.16, 138.45, 131.72, 127.48, 126.31, 121.34, 119.24, 116.12, 114.43, 112.89, 112.12, 100.76; Anal. Calcd.for $C_{15}H_{10}ClFN_2$: C, 66.70; H, 3.06; N, 10.27. Found: C, 65.82; H, 3.83; N, 10.41; IR (KBr, cm^{-1}): 3445.09, 3004.97, 1587.72, 1542.37, 1331.71, 1255.52.

Compound 1i: Yield=85%; mp=281-284 °C; Ms: m/z 283 $[M + 1]^+$; ^{1}H-NMR (300 MHz, $CDCl_3$ ppm): δ 2.18 (s, 3H, CH_3), δ 2.35 (s, 3H, CH_3), δ 8.29 (d, 1H, J=5.4 Hz, Ar-H), δ 8.14 (d, 1H, J=8.7 Hz, Ar-H), δ 7.91 (s, 1H, Ar-H), δ 7.45 (d, 1H, J=18.9 Hz, Ar-H), δ 7.06 (s, 1H, Ar-H), δ 7.06-7.09 (d, 3H, Ar-H + 1H, NH), δ 6.25 (d, 1H, J=5.4 Hz, Ar-H); ^{13}C-NMR (300 MHz, $CDCl_3$, ppm): δ 150.90, 150.83, 148.48, 137.33, 137.22, 135.99, 132.0, 131.44, 128.14, 127.68, 127.08, 126.03, 122.8, 117.65, 101.47, 20.91, 17.30; Anal. Calcd.for $C_{17}H_{15}ClN_2$: C, 72.24; H, 5.60; N, 9.76. Found: C, 72.41; H, 5.28; N, 10.21; IR (KBr, cm^{-1}): 348.63, 2593.15, 1578.07, 1445.69, 1207.25, 1093.78.

Compound 1j: Yield=82%; mp=166-168 °C; Ms: m/z 283 $[M + 1]^+$; ^{1}H-NMR (300 MHz, DMSO-d_6, ppm): δ 2.30 (s, 6H, CH_3), δ 8.46 (s, 1H, Ar-H), δ 7.83-7.90 (m, 3H, J=22.5, Ar-H + 1H, NH), δ 7.71 (d, 2H, J=8.4 Hz, Ar-H), δ 7.04-7.14 (m, 2H, Ar-H), δ 6.82 (d, 1H, J=9.6 Hz, Ar-H), ^{13}C-NMR (300 MHz, DMSO-d_6, ppm): δ 158.42, 149.96, 136.98, 135.41, 132.27, 131.83, 131.2, 131.0, 130.31, 129.99, 129.03, 127.34, 126.01, 124.8, 115.5, 19.72, 13.48; Anal. Calcd.for $C_{17}H_{15}ClN_2$: C, 72.44; H, 5.65; N, 9.96. Found: C, 72.41; H, 5.13; N, 10.20; IR (KBr, cm^{-1}): 3279.75, 2036.49, 1587.58, 1497.98, 1367.03, 1239.84.

Compound 1k: Yield=61%; mp>281-283 °C; Ms: m/z 323 $[M + 1]^+$; ^{1}H-NMR (300 MHz, DMSO-d_6, ppm): δ 8.90 (d, H, J=9.0 Hz, Ar-H), δ 8.58 (d, 1H,J=4.0 Hz, Ar-H), δ 8.24 (d, 1H, J=3.0 Hz, Ar-H), δ 7.78-7.81 (m, 3H, J=8.4 Hz, Ar-H + 1H, NH),δ 6.41 (d, 1H, J=4.0 Hz, Ar-H); ^{13}C-NMR (300 MHz, DMSO-d_6, ppm): δ 155.31, 144.42, 139.79, 138.92, 136.08, 133.22, 132.63, 130.5, 130.45, 129.96, 128.19, 126.58, 120.15, 116.19. 101.72; Anal.Calcd.for $C_{15}H_9Cl_3N_2$: C, 55.77; H, 2.70; N, 8.56. Found: C, 56.20; H, 3.12; N, 7.58.; IR (KBr, cm^{-1}): 3006.19, 2676.07, 1607.65, 1542.56, 1459.16, 1330.02.

Compound 1l: Yield=75%; mp=210-213 °C; Ms: m/z 291 $[M^+]$, ^{1}H-NMR (300 MHz, DMSO-d_6, ppm): δ 9.03 (d, 1H, H_2, J=9.0Hz), δ 8.62 (d, 1H, J=9.0 Hz), δ 8.26 (s, 1H, Ar-H), δ 7.86 (d, 1H,J=8.7 Hz, Ar-H), δ 7.67-7.53 (q, 2H, J=22.4 Hz, Ar-H), δ 7.32-7.37 (1H, NH); ^{13}C-NMR (300 MHz, DMSO-d_6, ppm): δ 155.44, 143.62, 138.0, 138.57, 133.83, 133.26, 132.36, 131.12, 130.41, 129.27, 127.83, 126.40, 19.41,115.70,100.77; Anal. Calcd. (%) for $C_{15}H_9ClF_2N_2$: C, 61.88; H, 3.22; N, 9.07. Found: C, 60.43, H, 4.12, N, 9.56; IR (KBr, cm^{-1}): 3006.54, 2886.07, 1605.65, 1512.52, 1451.13, 1330.22.

Compound 1m: Yield=87%; mp>305°C; Ms: m/z 273 $[M+ 1]^+$; ^{1}H-NMR (300 MHz, DMSO-d_6, ppm): δ 4.66 (s, 1H, NH), δ 8.87 (d, 1H, J=9.0 Hz), δ 8.61 (d, 1H, J=7.3 Hz), δ 8.20 (s, 1H, H_8), δ 7.84 (d, 1H,H_6 J=8.7 Hz), δ 7.34-7.62 (3H, Ar-H + 1H, NH), δ 7.03 (d, 1H, J=6.9 Hz, Ar-H); ^{13}C-NMR (300 MHz, CD_3OD, ppm): δ 157.25,143.55, 141.38, 141.29, 140.28, 137.51, 130.80, 129.05, 126.09, 123.91, 120.23, 117.01, 101.51; Anal. Calcd. (%) for $C_{15}H_{10}ClFN_2$: C, 66.75; H, 3.06; N, 10.22. Found: C, 66.43; H, 4.22; N, 10.41; IR (KBr, cm^{-1}): 3184.15, 3115.37, 2649.47, 1585.09, 1540.87, 1291.49.

Compound 1n: Yield=82%; mp=314-317 °C; Ms: m/z 283 $[M+ 1]^+$; ^{1}H-NMR (300 MHz, $CDCl_3$, ppm): δ 2.28 (s, 6H, CH_3), δ 8.51 (d, 1H,J=8.7 Hz, Ar-H), δ 8.16 (d, 1H, J=6.9 Hz, Ar-H), δ 7.92 (s, 1H, Ar-H), δ 7.54 (s, 1H, Ar-H), δ 7.12 (s, 1H, NH), δ 6.73 (d, 1H, J=6.9 Hz, Ar-H); ^{13}C-NMR (300 MHz, $CDCl_3$, ppm): δ 155.66, 141.44, 139.96, 138.61, 138.33, 136.44, 133.46, 130.59, 127.63, 125.73, 124.54, 122.13, 118.73, 115.31, 99.76, 18.87, 18.48; Anal. Calcd. (%) for $C_{17}H_{15}ClN_2$: C, 72.20; H, 5.45; N, 9.82. Found: C, 73.12; H, 4.89; N, 8.12; IR (KBr, cm^{-1}): 3370.21, 2834.24, 1612.59, 1408.51, 1365.66, 1229.94.

Compound 1o:Yield=75%; mp=195 °C; Ms: m/z 314 $[M^+]$; ^{1}H-NMR (300 MHz, DMSO-d_6, ppm): δ 3.74 (s, 6H, OCH3), δ 8.86 (d, 1H,J=9.0 Hz, Ar-H), δ 8.48 (d, 1H, J=6.9 Hz, Ar-H), δ 8.21 (s, 1H, Ar-H), δ 7.84 (d, 1H, J=9.0 Hz, Ar-H),), δ 7.04 (s, 1H, NH), 7.07 (s, 1H, Ar-H), δ 6.35 (d, 1H,

J=6.9 Hz, Ar-H), ^{13}C-NMR (300 MHz, DMSO-d_6, ppm): δ 155.19, 153.45, 148.26, 142.85, 138.92, 138.25, 127.28, 126.07, 125.19, 119.22, 115.48, 114.39, 113.9, 113.69, 101.078, 56.08, 55.67; Anal. Calcd.for $C_{17}H_{15}N_2O_2$: C, 64.77; H, 4.90; N, 8.70. Found: C, 65.13; H, 5.20; N, 7.75; IR (KBr, cm^{-1}): 3370.21, 3057.92, 1612.59, 1560.07, 1365.66, 1313.57.

Compound 1p: Yield=71%; mp=277-279 °C; Ms: m/z 306 [M^+]; ^{1}H-NMR (300 MHz, $CDCl_3$, ppm): δ 9.52 (d, 1H, J=8.7 Hz, Ar-H), δ 9.17 (d, 1H, J=7.2 Hz,Ar-H), δ 8.93 (s, 1H, Ar-H), δ 8.65 (d, 1H, J=9.3 Hz, Ar-H), δ 8.56 (s, 1H, Ar-H), δ 8.25 (d, 1H, J=7.5 Hz, Ar-H), δ 8.07-8.13 (m, 3H,J=1.5 Hz, Ar-H + 1H, NH), δ 7.75 (d, 1H, Ar-H); ^{13}C-NMR (300 MHz, $CDCl_3$, ppm): δ 155.29, 143.64, 138.96, 138.67, 133.83, 133.26, 132.36, 131.12, 130.41, 129.27, 127.83, 126.14, 119.41, 115.61, 101.03; Anal. Calcd.for $C_{15}H_9Cl_2FN_2$: C, 58.95; H, 2.66; N, 9.19. Found: C, 57.30; H, 3.32; N, 10.41; IR (KBr, cm^{-1}): 3279.75, 2809.57, 2036.49, 1615.49, 1539.97, 1337.05.

4.4 General procedure for synthesis of various N-benzyl-N'-(7-chloroquinolin-4-yl)-ethane-1, 2-diamine (2a-2l)

In a round bottom flask, added *N*^{1}-(7-chloroquinolin-4-yl)-ethane-1, 2-diamine (13-15 mmol) and aldehydes (10 mmol) under nitrogen atmosphere in dry acetonitrile (30-40 mL). Allow the reaction mixture at stirring for 2-4 h at room temperature. Progress of reaction was monitored by thin layer chromatography. When the spot of aldehyde and amine get disappeared in TLC, 15-17 mmol of sodium triacetoxyborohydride in dry CH_3CN (5 mL) or dry DCM (5 mL) (depending on the aldehydes used) was added into the reaction mixture under a nitrogen atmosphere.The reaction mixture was left for 18-23 h on constant starring at room temperature and progress of reaction was monitored by TLC. Imine formation, however, is a reversible reaction and requires long reaction times and the use of a dehydrating agent such as molecular sieves can be used for the reaction to completion. After completion of the reaction, the reaction mixture was extracted with ether (3 x 10 mL). The combined ether extracts were concentrated under reduced pressure. The crude product thus obtained was purified by column chromatography (silica gel 60-120 mesh) using ethylacetate-hexane as eluent [33, 34].

Characterization data

Compound 2a: Yield=62%; Brown oily compound; Ms: m/z 341 [M^+]; ^{1}H-NMR (300 MHz, CD_3OD, ppm): δ 2.62 (s, 2H, CH_2), δ 3.65 (d, 2H, J= 3.3 Hz, CH_2), δ 3.70 (s, 3H, OCH_3), δ 3.81 (d, 2H, J = 5.2 Hz, CH_2), δ 8.87 (d, 1H, J=8.1 Hz, Ar-H), δ 8.24 (s, 1H, Ar-H), δ 7.36 (d, 1H, J=7.3 Hz,

Ar-H), δ 6.33 (d, 1H, J=7.1 Hz, Ar-H), δ 6.54-6.88 (m, *J*=10.2 Hz, 1H, NH + 2H, Ar-H,), δ 7.16-6.94 (m, 3H, J = 20.4,Ar-H +1H, NH); ^{13}C-NMR (300 MHz, CD_3OD, ppm): δ 158.42, 150.21, 149.59, 143.10, 141.47, 136.18, 134.83, 130.52, 129.21, 121.15, 120.82, 119.22, 111.10, 110.29, 103.41, 62.40, 58.71, 44.72, 38.14;Anal. Calcd.for $C_{19}H_{20}ClN_3O$: C, 68.56; H, 5.82; N, 15.49. Found. C: 68.95, H: 5.45, N: 15.15; IR (KBr, cm^{-1}): 3327.34, 2626.07, 1815.65, 1563.56, 1445.32, 1361.03.

Compound 2b: Yield=86%; mp=158-161 °C; Ms: m/z 380 [M^+]; ^{1}H-NMR (400 MHz, DMSO-d_6, ppm): δ 3.31 (s, 2H, CH_2), δ 3.60 (d, 2H, J= 5.4 Hz, CH_2), δ 3.86 (d, 2H, J = 5.7 Hz, CH_2), δ 8.39 (d, 1H, J=5.4 Hz, Ar-H), δ 8.34 (s, 1H, Ar-H), δ 7.75 (t, 1H, J=6.9 Hz, Ar-H), δ 7.51 (s, 1H, Ar-H), δ 8.25 (s, 1H, NH), δ 8.22 (s, 1H, NH);^{13}C-NMR (300 MHz, DMSO-d_6, ppm): δ149.49, 142.35, 138.35, 137,26, 137.15, 134.48, 130.19, 125.46, 122.23, 118.05, 114.67, 110.18, 106.19, 105.22, 103.89, 55.43, 48.77, 46.30; Anal. Calcd.for $C_{18}H_{16}Cl_3N_3$: C, 68.89; H, 7.24; N, 15.14. Found: C, 68.72; H, 7.28; N, 15.21; IR (KBr, cm^{-1}): 3316.19, 2453.43, 1627.55, 1616.24, 1320.02, 1243.45.

Compound 2c: Yield=71%; mp=147-150°C; Ms: m/z 390 [M^+]; ^{1}H-NMR (400 MHz, DMSO-d_6, ppm): δ 2.69 (s, 2H, CH_2), δ 3.61 (d, 2H, CH_2, J = 4.5 Hz), δ 3.87 (d, 2H, CH_2, 3.9 Hz), δ 8.51 (br, s, 1 H, NH), δ 8.38 (d, 1H, J = 4.5, Hz Ar-H), δ 8.22 (d, J=7.5 Hz, 1H, Ar-H), δ 7.76 (d, 1H, J = 2.7 Hz, Ar-H), δ 7.44 (d, 1H, Ar-H), δ 6.84 (d, 2H, Ar-H+ NH), δ 6.59 (d, 1H,*J*= 4.2 Hz, Ar-H); ^{13}C-NMR (400 MHz, $CDCl_3$, ppm): δ 168.54, 159.40, 151.85, 149.84, 148.89, 136.67, 130.9, 129.54, 128.50, 128.17, 125.30, 121.24, 99.17, 50.23, 46.63, 41.82; Anal. Calcd.for $C_{18}H_{17}BrClN_3$: C, 55.33; H, 4.39; N, 10.07. Found: C, 56.35; H, 4.30; N, 11.20; IR (KBr, cm^{-1}): 3236.19, 2903.43, 1617.65, 1566.24, 1330.02, 1243.00.

Compound 2d: Yield=67%; mp=148-149°C; Ms: m/z 329.11 [M^+]; ^{1}H-NMR (400 MHz, DMSO-d_6, ppm): δ 2.62 (s, 2H, CH_2), δ 3.83 (d, 2H, J = 4.6 Hz, CH_2), δ 3.83 (d, 2H, J = 3.7 Hz,CH_2), δ 9.21 (s, 1H, NH), δ 8.89 (d, 1H, J=8.0 Hz, Ar-H), δ 8.34 (s, 1H, NH), δ 7.66 (d, 1H, J=5.3 Hz, Ar-H), δ 7.35 (d, 1H, J=5.9 Hz, Ar-H), δ 6.31 (d, 1H, J=4.2 Hz, Ar-H), δ 6.55 (d, 1H, J=6.2 Hz, Ar-H); ^{13}C-NMR (400 MHz, $CDCl_3$, ppm): δ 160.71, 158.52, 151.20, 149.23, 147.34, 144.15, 136.21, 133.22, 131.10, 130.82, 129.20, 121.45, 112.20, 103.42, 59.10, 48.23, 35.51;Anal. Calcd.for $C_{18}H_{17}FClN_3$: C, 66.55; H, 5.20; N, 12.74. Found: C, 65.83; H, 5.58; N, 13.20. IR (KBr, cm^{-1}): 3162.27, 1620.88, 1467.01, 1381.13, 1292.77.

Compound 2e: Yield=50%; mp=227-232°C; Ms: m/z 345.08 [M^+]; ^{1}H-NMR (400 MHz, $CDCl_3$, ppm): δ 2.62 (s, 2H, CH_2), δ 3.25 (d, 2H, J = 3.6,CH_2), δ 3.80 (d, 2H, J = 4.1,CH_2), δ 9.31 (s, 1H, NH), δ 8.82 (d, 1H, J=8.1 Hz, Ar-H), δ 8.33 (s, 1H, Ar-H), δ 7.35 (d, 1H, J=5.1 Hz, Ar-H), δ 6.32 (d, 1H, J=4.2 Hz, Ar-H), δ 6.56 (s, 1H, J=3.2 Hz, NH); ^{13}C-NMR (400 MHz, $CDCl_3$, ppm): δ 151.58, 149.10, 147.45, 144.92, 136.18, 134.62, 132.24, 131.10, 130.81, 129.22, 121.32, 112.21, 103.14, 48.72, 36.92, 32.51; Anal. Calcd.for $C_{18}H_{17}Cl_2N_3$: C, 62.44; H, 4.95; N, 11.14. Found: C, 63.24; H, 5.18; N, 10.15; IR (KBr, cm^{-1}): 3143.27, 1667.81, 1462.01, 1392.23, 1207.12.

Compound 2f: Yield=88%; mp=219-221°C; Ms: m/z 336 [M^+]; ^{1}H-NMR (400 MHz, DMSO-d_6, ppm): δ 2.44 (s, 2H, CH_2), δ 3.53 (d, 2H, J = 4.5 Hz, CH_2), δ 3.76 (d, 2H, J = 4.8 Hz, CH2), δ 8.38 (d, 1H, J=4.9 Hz, Ar-H), δ 8.22 (d, 1H, J = 6.6, Ar-H), δ 8.10 (s, 1H, J = 6.6, Ar-H), δ 7.76 (d, 1H, J=1.5 Hz, Ar-H), δ 7.43-7.41 (m, 2H, J = 8.1, Ar-H), δ 7.17 (d, 1H, J = 1.2, Ar-H), δ 6.92 (d, 1H, Ar-H), δ 6.33 (s,br, 1H,NH), δ 5.28 (s, br, 1H, NH); ^{13}C-NMR (400 MHz, $CDCl_3$, ppm): δ160.91, 150.20, 149.49, 142.35, 138.55, 137.26, 137.15, 134.48, 130.19, 122.23, 118.05, 110.1, 106.19, 105.22, 48.77, 30.92, 24.76; Anal. Calcd. (%) for $C_{19}H_{17}ClN_4$: C, 67.75; H, 5.09; N, 16.53. Found: C, 66.89; H, 5.15; N,17.30; IR (KBr, cm^{-1}): 3108.27, 1621.24, 1462.01, 1341.20, 1307.12.

Compound 2g: Yield=62%; mp>133-136°C; Ms: m/z 345 [M + 1]; ^{1}H-NMR (300 MHz, DMSO-d_6, ppm): δ 3.18 (d, 2H, J = 4.9, CH_2), δ 3.60 (d, 2H, J = 4.5Hz, CH_2), δ 3.18 (s, 2H, CH_2), δ 8.39 (d, 1H, *J*=4.2 Hz), δ 8.33 (s, 1H, NH), δ 7.77 (s, 1H, NH), δ 6.58 (d, 1H, J = 4.5Hz, Ar-H); ^{13}C-NMR (400 MHz, $CDCl_3$, ppm): δ 168.66, 150.98, 144.60, 138.57, 134.55, 130.10, 129.94, 129.58, 128.37, 127.86, 126.75, 126.56, 125.97, 124.52, 119.67, 50.73, 46.86, 41.42; Anal. Calcd.for $C_{18}H_{17}Cl_2N_3$: C, 62.19; H, 4.98; N, 12.34. Found: C, 62.04; H, 5.20; N, 11.74; IR (KBr, cm^{-1}): 3125.89, 1786.22, 1542.12, 1299.02.11, 1297.79.

Compound 2h: Yield=34%; mp=210-212°C; Ms: m/z 371; ^{1}H-NMR (400 MHz, $CDCl_3$, ppm): δ 3.20 (s, 2H, CH_2), δ 3.60 (s, 6 H, OCH_3), δ 3.85 (d, 2H, J = 3.2 Hz, CH_2), δ 4.12 (d, 2H, J = 4.9 Hz, CH_2), δ 8.87 (d, 1H, J=8.1 Hz, Ar-H), δ 8.22 (s, 1H, Ar-H), δ 7.75 (s, 1H, NH), δ 7.33 (d, 1H, J=7.1 Hz, Ar-H), δ 6.53 (s, 1H, NH), δ 6.19 (s, 1H, Ar-H), δ 6.09 (d, 1H, J = 5.8Hz, Ar-H);^{13}C-NMR (400 MHz, $CDCl_3$, ppm): δ 163.4, 160.2, 151.5, 147.0, 144.7, 135.1, 133.1, 130.8, 130.2, 124.2, 121.9, 112.2, 103.4, 57.1, 48.7, 41.80, 35.52; Anal. Calcd.for $C_{20}H_{22}ClN_3O_2$: C, 64.56; H, 6.00; N, 11.30.Found: C, 64.20; H, 5.62; N, 12.14; IR (KBr, cm^{-1}): 3336.27, 1660.83, 1487.66, 1288.61,1207.12.

Compound 2i: Yield=55%; mp=217-220°C; Ms: m/z 326 [M^+]; ^{1}H-NMR (400 MHz, $CDCl_3$, ppm): δ 2.39 (s, 3H, CH_3), δ 3.34 (s, 2H, J = 3.34, CH_2), δ 3.65 (s, 2H, J = 3.9, CH_2), δ 3.96 (t, 3 H, J = 7.8 Hz, CH_2), δ 5.51 (s, br, 1H, NH), δ 8.30 (s, 1H, J=8.0 Hz, Ar-H), δ 7.97 (d, 1H, J = 1.5Hz, Ar-H), δ 7.66-7.69 (m, 1H, J = 9.9Hz, Ar-H), δ 6.93 (s,br, NH); ^{13}C-NMR (400 MHz, $CDCl_3$, ppm): δ144.69, 142.10, 141.07, 132.11, 131.04, 129.88129.68, 129.60, 128.45, 128.27, 120.70, 18.94, 113.90, 55.24, 41.21, 29.67, 21.51; Anal. Calcd.for $C_{19}H_{20}ClN_3$: C, 70.94; H, 6.18; N, 12.00. Found: C, 68.26; H, 5.58, N, 14.60; IR (KBr, cm^{-1}): 3466.57, 1710.88, 1432.01, 1324.87,1243.12.

Compound 2j: Yield=48%; mp=228-231°C; Ms: m/z 347 [M^+]; ^{1}H-NMR (400 MHz, $CDCl_3$, ppm): δ 3.32 (s, 2H, CH_2), δ 3.68 (d, 2H, J = 4.2Hz, CH_2), δ 4.03 (d, 2H, J = 3.9Hz, CH_2), δ 8.77 (s, 1 H, NH), δ 8.55 (d, 1H, J=4.5 Hz, Ar-H), δ 7.95-8.02 (m, 3H, Ar-H), δ 7.69 (s, 1 H, Ar-H), δ 7.26 (s, 1 H, NH), ^{13}C-NMR (400 MHz, $CDCl_3$, ppm): δ 161.52, 151.90, 149.62, 149.04, 142.04, 134.91, 133.97, 130.78, 130.23, 128.64, 125.41, 121.04, 99.30, 59.07, 43.59, 30.89; Anal. Calcd.for $C_{18}H_{16}ClF_2N_3$: C, 62.64; H, 4.16; N, 12.08. Found: C, 63.14; H, 4.48; N, 11.20; IR (KBr, cm^{-1}): 3006.54, 2886.07, 1605.65, 1512.52, 1451.13, 1330.22.

Compound 2k: Yield=41%; mp>211-213°C; Ms: m/z 371.14 [M^+]; ^{1}H-NMR (400 MHz, $CDCl_3$, ppm): δ 2.48 (s, 2H, CH_2), δ 2.85 (d, 2H, J = 7.2Hz, CH_2), δ 3.45 (d, 2H, J = 7.7, CH_2), δ 8.25 (d, 1 H, J=6.6 Hz, Ar-H), δ 8.01 (d, 1 H, J=6.3 Hz, Ar-H), δ 7.64-7.72 (m, 5H, Ar-H + 1H, NH), δ 7.60 (s, 1H, NH), δ 6.32 (d, 1 H_6, *J*=7.1 Hz), δ 6.53 (d, 1 H_3, *J*=7.5 Hz), δ 6.19 (s, 2 H, Ar-H), δ 6.09 (s, 1H, Ar-H). ^{13}C-NMR (400 MHz, $CDCl_3$, ppm): δ 189.09, 168.49, 161.63, 150.96, 139.76, 138.51, 132.69, 129.54, 128.52, 127.28, 127.10, 126.72, 119.56, 98.94, 60.80, 39.79, 30.90, 24.56; Anal. Calcd. (%) for $C_{20}H_{22}ClN_3O_2$: C, 61.53; H, 6.30; N, 14.36. Found: C, 60.22; H, 5.98; N, 15.65; IR (KBr, cm^{-1}): 34548.63, 2673.43 1570.07, 1541.61, 1233.59, 1247.30.

4.5 *In vitro* susceptibility assay of *P. falciparum*

Antiplasmodial activity of compounds was determined against erythrocytic stages of the CQ (100 ng/mL), pyrimethamine (15 nM) and cycloguanil resistant FCR3 strain of *P. falciparum* by a modified [3H]-hypoxanthine incorporation assay [35]. Briefly, all parasites were cultured in 96-well microtitre plates and incubated at 37 °C under 2% O_2, 5.5% CO_2, 92.5% N_2 atmosphere in RPMI 1640 (Invitrogen) with 25mM HEPES, 25mM $NaHCO_3$, 200mM L-glutamine, 50mg/L gentamycin (Gibco), 5g/L Albumax II (Life Technologies) and 20mg/L tritium labeled hypoxanthine (Perkin Elmer) with a final reactivity of 0.01 µCi. Parasite cultures were grown and

synchronized to ring stages and used at 1% parasitemia at 2% haematocrit of blood group O erythrocytes. Cultures were exposed during 48 hours of incubation where after the plates were harvested onto a filter plate (Perkin Elmer) using a Filter Mate harvester (Perkin Elmer). To each well 50 µl of Microscint 20 (Sigma) was added and the plate counted in a MXT top count (Perkin Elmer). Experiments included untreated controls and serial compound dilutions covering a range from 0.05 mg/ml to $2x10^{-6}$ mg/ml, tested in triplicate and in two independent assays. As a control for parasite inhibition we used artesunate (IC_{50} = 0.91µg/L± 0.17µg/L). The IC_{50} values were calculated from the dose-response curves using GraphPad Prism software.

4.6 *In vivo* efficacy of amodiaquine derivatives against *Plasmodium berghei*

Evaluation of the curative potential of the few selected amodiaquine derivatives was done using the method described by Ryley and Peters, 1970 (rodent malaria four-day suppressive test; Peters' four-day suppressive test) [36, 37]. Rodent malaria parasite *Plasmodium berghei* ANKA chloroquine resistant strain, obtained from National Institute of Malaria Research (NIMR), Delhi was used for *in vivo* studies.

4.6.1 Experimental animals: Naïve Balb/C mice, males, 25 ± 5g (4-5 week old) free from *Eperythrozoon coccoides* and *Haemobartonella muris*, were obtained from the Animal Facility Centre, Department of Zoology, University of Delhi, Delhi, India. They were maintained on a commercial pellet diet and housed under appropriate conditions (room temperature at 22±2°C and 50-60% relative humidity, diet with *p*-aminobenzoic acid content of 45mg/kg, and water *ad libitum.)*. The study was conducted in accordance with the internationally accepted principles for laboratory animal use and care.

4.6.2 Drug solutions: Each compound was made to strength of 2 mg/ml stock solution in 10% DMSO and administered according to desired concentration and individual body weight.

4.6.3 Test Procedure: On **Day 0**, heparinized blood was withdrawn from an infected donor mouse with approximately 25-30% parasitemia, and diluted in physiological saline to 10^8 parasitized erythrocytes per ml. An aliquot of 0.2ml (=$2x10^7$ parasitized erythrocytes) of this suspension was injected intraperitoneally (i.p.) into experimental groups of 5 mice each. One to

three hours post-infection, the experimental groups were treated with varying doses of each of the test compounds (0.1, 1, 5, 10 mg/kg BW) by the ip route. Identical doses of amodiaquine were administered to the standard drug group and 0.2 mL of normal saline to the negative control group.On **Days 1 to 3**, i.e. 24hrs, 48hrs and 72hrs post-infection, the experimental groups of mice were treated again with the same dose and by the same route as on day 0. 24Hrs after the last treatment, on **Day 4** (i.e. 96 hrs post-infection), blood smears from the tail region of mice were made and stained with Giemsa stain for microscopic study by counting 4 fields of approximately 300-500 erythrocytes per slide, to assess antimalarial efficacy of the test compound.

Differences in parasitemia percentage between treated group animals and untreated animals were analyzed by a one-way ANOVA test using IBM SPSS Statistics 16.0 and differences considered significant if $P< 0.05$. Further, difference between the mean value of the control group (taken as 100%) and those of the experimental groups is calculated and expressed as percent reduction (= activity) using the following equation:

Mean parasitemia treated Activity = 100 – (Mean parasitemia/control) x 100

In untreated control mice mortality was observed approximately one week after infection. Treated mice were observed for a period of 30 days, and the survival time (in days) was recorded. The mean survival time was calculated in comparison to untreated (Normal saline) and standard drug (amodiaquine) treated groups. Differences in survival time between treated groups and untreated animals were analyzed by Log-rank (Mantel-Cox) test using GraphPad Prism 5 and differences considered significant if $P <0.005$. Observations concerning adverse effects due to the compounds were recorded.

4.7 Cytotoxicity against Huh-7 cells

Cytotoxicity of the compounds was evaluated in human hepato-cellular carcinoma cells (Huh-7) using MTT assay [38] and CC_{50} values were calculated.Assays were performed in 96-well microtiter plates, each well containing 100 μL of DMEM medium supplemented with 1% penicillin-streptomycin-glutamine solution and 10% fetal bovine serum, and 4×10^3 Huh-7 cells. Serial drug dilutions of eight 2-fold dilution steps covering a range from 100 to 0.78μg / mL were prepared. After 72 h of incubation the plates were inspected under an inverted microscope to assure growth of the controls and sterile conditions. 10 μl of MTT reagent (5 mg MTT dissolved in 1 mL PBS) was then added to each well and the plates incubated for 2-4 h in the cell culture

incubator. 100 µl of detergent reagent (90% isopropanol, 9.999% triton x-100, 0.001% conc. HCl) was then added to each well and the plates incubated for another 2 h in the dark at room temperature. The absorbance in each well was read with a Biotek Synergy HT microplate reader at a wavelength of 570 nm. Data were analyzed using the microplate reader software. Each CC_{50} value obtained is the mean of at least two separate experiments performed in duplicate. Amodiaquine was the standard drug used.

4.8 Therapeutic Index in mouse model

LD_{50} test was carried out on BALB/c mice using different dosages of various compounds: 50, 100, 200, 500, 600, 800 and 1000mg/kg BW ip and the animals were observed for 7 days. Therapeutic Index (TI) values were determined by the formula:

$$\text{Therapeutic Index (TI)} = \frac{\text{Median lethal dose } (LD_{50})}{\text{Median effective dose } (ED_{50})}$$

4.9 Docking Energy Calculations

The 3D optimized structure of dimeric hematin was prepared using Marwin Sketch and Gaussian 09W software [39, 40]. For the optimization of ligands (amodiaquine derivatives viz. 1m, 1o, 2c and 2j), we used 6-31 G basis set, Hartee-Fock method, default spin-singlet, ground state and zero charge in Gaussian 09W software. Autodock tools 1.5.6 with AutoGrid 4 and AutoDock4 were used to set up and perform docking calculations [41, 42]. To visualize the docking results, we used Pymol, an open-source projected maintained by DeLano Scientific LLC and Viewerlite 4.2 software (Accelrys, San Diego, CA, USA).

The 3D structure of PfLDH complexed with NADH and the substrate oxamate used in the present study was obtained from the Protein Data Bank (PDB) file 1LDG [43]. We performed rigid docking studies with the four amodiaquine derivatives, aimed at drawing a comparison of their interactions with that of protonated chloroquine at the active site of PfLDH [22]. For each of the docking cases, the lowest energy docked conformation, according to the autodock scoring function, was selected as the binding mode.

Abbreviations

ACT: Artemisinin-based combined therapy, RPMI: Roswell Park Memorial Institute (cell culture medium), HEPES: 4-(2-hydroxyethyl)-1-piperazine-ethanesulfonic acid, IC_{50}: Median inhibition concentration of growth, ED_{50}: Median effective dose, DMSO: Dimethyl sulphoxide, MST: mean survival time, SAR: Structure-activity relationship, BW: Body weight, $CC_{50=}$ Cytotoxic concentration 50, TI= Therapeutic index, TMS = Tetramethylsilane.

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Ethical approval: Yes

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Highlights

- We propose the synthesis of amodiaquine analogues.
- Antiplasmodial potential was assessed over chloroquine-resistant parasite strains, *in vitro* and *in vivo*.
- Mode of action was elucidated *in silico.*
- Selected analogues exhibit superior antiplasmodial profile.