

## Synthesis and *in vitro* antiplasmodial evaluation of some isoquine analogues

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### ABSTRACT

Isoquine is the regioisomer of amodiaquine that can not form hepato and haematotoxic active metabolites by biotransformation unlike amodiaquine. In present investigation, five new isoquine analogues were synthesized by a two-step method, characterized by spectral analyses and subsequently evaluated *in vitro* against the chloroquine sensitive RKL-2 strain of *Plasmodium falciparum* by JSB staining method. All synthesized compounds demonstrated differential extent of antiplasmodial activity against the test parasites. However, no compound was found to exhibit better antiplasmodial profile as compared to chloroquine.

**Key words:** Isoquine, antiplasmodial, RKL-2, *Plasmodium falciparum*, JSB staining.

### INTRODUCTION

Malaria is a vector-borne infectious disease caused by protozoan parasites. It is widespread in tropical and subtropical regions, including parts of the Americas, Asia, and Africa. Each year, there are approximately 350-500 million cases of malaria, killing between one and three million people, the majority of whom are young children in Sub-Saharan Africa. Malaria is a leading cause of morbidity and mortality in developing world (Snow *et al.*, 2005). The disease is caused by protozoan parasites of the genus *Plasmodium*. Chloroquine was a mainstream drug active against *Plasmodium falciparum*, but its efficacy has been diminished by the emergence of chloroquine-resistant parasites. The spread of chloroquine resistance has prompted the re-investigation of the chemistry and pharmacology of alternative antimalarials such as amodiaquine, an other 4-aminoquinolines which proved to be effective against

chloroquine-resistant strains (Anonymous, 1997; Olliaro *et al.*, 1996).

Amodiaquine is a 4-aminoquinoline antimalarial agent which is effective against many chloroquine resistant strains of *P. falciparum*. However, clinical use of amodiaquine has been severely restricted because of hepatotoxicity and agranulocytosis (Lind *et al.*, 1973; Neftel *et al.*, 1986). It was suggested that the toxicity of amodiaquine is due to the reactive electrophilic metabolites formed by biotransformation or bioactivation, causing oxidation of its phenolic side chain, leading to the formation of a quinoneimine by cytochrome P-450-catalyzed biological oxidation (Scheme 1). It was found that amodiaquine is excreted in bile exclusively as the 5' thioether conjugates (glutathione and cysteinyl) in rats (Harrison *et al.*, 1992). This observation indicated that the parent drug undergoes extensive bioactivation *in vivo* to form amodiaquine

quinoneimine (AQQI) or semiquinoneimine (AQSQI) with subsequent glutathione conjugation (Maggs *et al.*, 1988).

Structure activity relationship (SAR) studies on amodiaquine previously showed that wide variations in the side chain can be accommodated with retention of antiplasmodial activity. Blocking of bioactivation pathways by removal of the phenolic group or introduction of non reactive substituents has been the main strategy. Reducing bioactivation also seems to result in compounds with slower elimination (enhanced biological half life), and increased tissue accumulation (Casteel, 2003).

From SAR studies it was noted that in the amodiaquine and tebuquine series of 4-aminoquinoline analogues, the presence of the 4' hydroxyl group within the aromatic ring imparts greater inherent antiplasmodial activity against chloroquine resistant parasites than the corresponding deoxo analogues (O'Neill *et al.*, 1997; 1998). Interchange of the hydroxyl group and the Mannich side chain provides a means of preventing oxidation to toxic metabolites while retaining possible important bonding interactions with the aromatic hydroxyl function. This amodiaquine regioisomer i.e. isoquine can not form toxic metabolites by simple oxidation and is potent against chloroquine resistant parasites *in vitro* (Scheme 2). Isoquine itself was reported to possess potent *in vitro* and oral *in vivo* antimalarial activity in experimental animal models and it does not undergo *in vivo* biotransformation to quinoneimine metabolites (O'Neill *et al.*, 2003). Apart from an excellent antiplasmodial profile, isoquine and its different side-chain analogues are rather inexpensive antimalarials to synthesize and may represent new leads for development of safe, cheap, affordable, and effective antimalarials for both prophylaxis and treatment of malaria. Considering the aforesaid facts we have been designing, synthesizing and evaluating different new isoquine analogues. The present paper aims to report the synthesis and *in vitro* antiplasmodial evaluation of five of those compounds (Table 1).

### Chemistry

The synthesis of designed isoquine analogues involved a two-step procedure according to the method originally utilized by Burkhalter and

colleagues with minor modifications (Burkhalter *et al.*, 1948).

### Step I

This step involved a Mannich reaction of 3-hydroxyacetanilide with primary amines to provide the Mannich product (intermediate) in yields ranging from 50% to 90% (Scheme 3).

### Step II

This step involved the hydrolysis of the amide function to provide the corresponding Mannich-substituted 3-aminophenol which was subsequently coupled with 4, 7-dichloroquinoline (Scheme 4) to provide the target compounds shown in Table 1.

## EXPERIMENTAL

### Reagents and chemicals

4,7-dichloroquinoline was obtained from Mangalam Drug and Organics Ltd., Mumbai, India, as gift sample. Chloroquine was obtained from Glenmark Pharmaceuticals Ltd., Nashik, India. All the other chemicals used were of synthetic grade chemicals of Aldrich and Rankem, without further purification and obtained from commercial suppliers.

### General experimental procedures

The completion of reactions was tested by analytical thin layer chromatography on aluminum sheets pre-coated with silica gel obtained from Merck. Visualization was attempted by iodine vapour and UV light. Melting point of the synthesized compounds was determined on Veego, Model No. MPI, by open capillary method. The UV absorption maxima ( $\lambda_{max}$ ) were recorded on Shimadzu UV-1700 UV-Visible spectrophotometer. The FTIR spectra were recorded on Hitachi 270-50 spectrophotometers using potassium bromide pellets. The  $^1\text{H-NMR}$  and  $^{13}\text{C-NMR}$  spectra in DMSO were recorded at 400 MHz and 100 MHz respectively by Bruker 400 NMR spectrometer. Chemical shift values were given in  $\delta$  (ppm) scale using TMS as an internal standard. Significant  $^1\text{H-NMR}$  data were presented in order: number of protons, multiplicity (b, broad; s, singlet; d, doublet; t, triplet; m, multiple), coupling constants in hertz, assignment. The mass spectra of the synthesized compounds were recorded on Waters Micromass

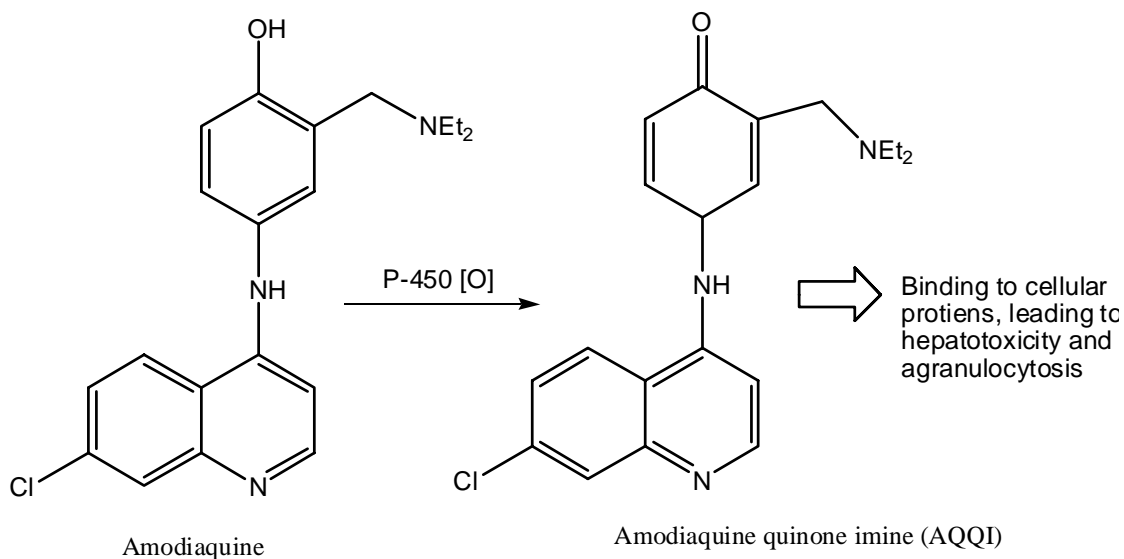
Q-ToF Micro Mass spectrometer. The  $m/z$  values of the more intense peaks are mentioned.

### Synthesis of the designed compounds

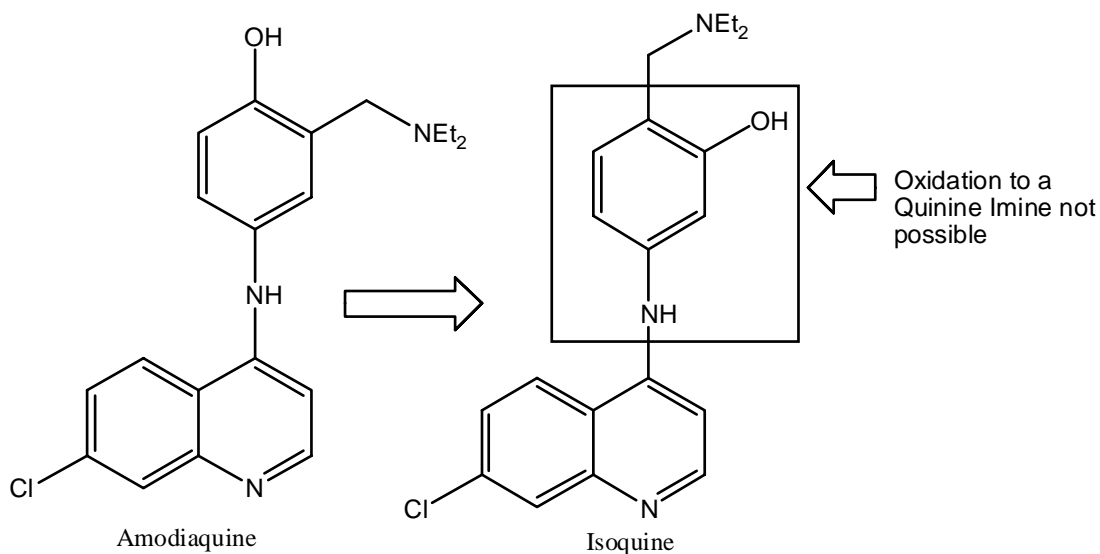
All the designed compounds (CS-8 to CS-12) were synthesized as per the schemes described in step-I & step-II of synthesis by using the following method.

### Step I

Ethanol was added to 3-Hydroxyacetanilide in a 100 ml round-bottom flask followed by one equivalent of primary or secondary amine and aqueous formaldehyde was added and the solution was allowed to heat under reflux for 24 hours. After this reflux period, the solvent was



**Scheme 1: Bioactivation of amodiaquine to toxic quinoneimine metabolite by P-450**



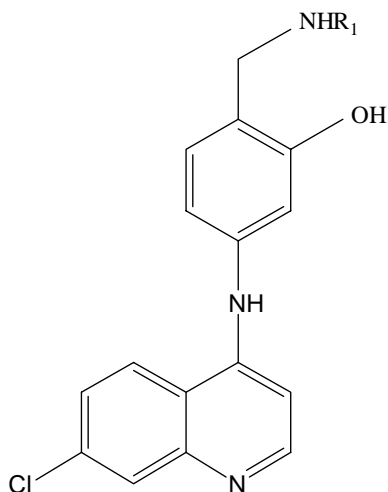
**Scheme 2: Redesign of Amodiaquine**

removed under reduced pressure and the crude material (intermediate amide) was purified by flash column chromatography using 20-80% MeOH/dichloromethane as eluent.

### Step II

Aqueous hydrochloric acid (20 %) (25 ml) was added to a round-bottom flask containing the amide (intermediate) and the solution was heated under reflux for 6 hours. The solvent was then removed *in vacuo* and the resulting residue co-evaporated with ethanol to give the corresponding hydrochloride salt. 4,7-dichloroquinoline and ethanol

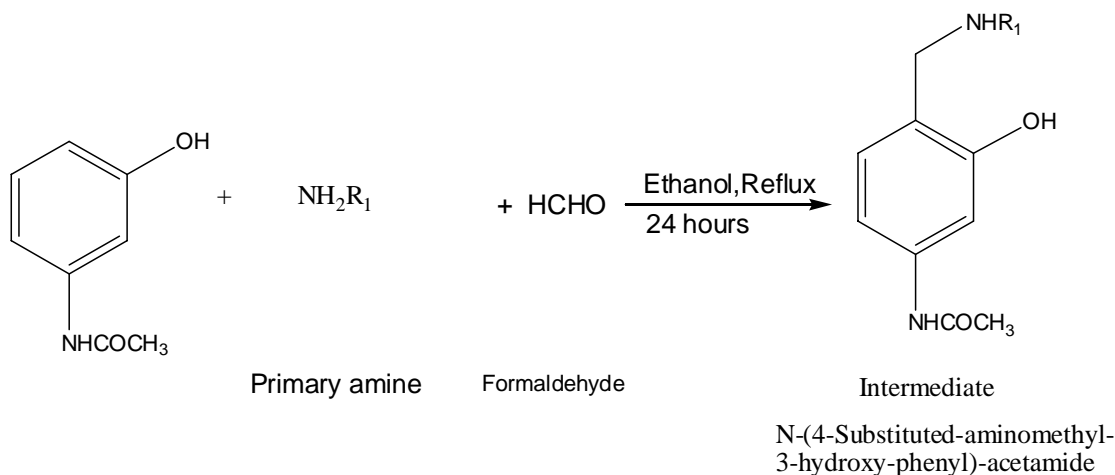
(30 ml) were added, and the reaction mixture was heated under reflux for around 12 hours until completion of the reaction (checked by analytical TLC). A crude product was obtained upon removing the solvent under reduced pressure; this was subsequently purified by flash column chromatography using 20-80% MeOH/dichloromethane as eluent to yield the quinoline hydrochloride salt. To liberate the free base compound, this solid was dissolved in distilled water (18 ml) and the solution was basified by careful drop wise addition of saturated sodium bicarbonate (added until no more precipitate was formed). Dichloromethane (100 ml) was added, and the free base was extracted into the organic layer. Subsequent drying and removal of solvent *in vacuo* at 45 °C afforded the desired product.



**Fig. 1: General structure of designed isoquine analogues**

### CS-8: 5-(7-chloroquinolin-4-ylamino)-2-[(2-fluoro phenylamino) methyl] phenol

CS-8 was obtained as brownish yellow solid (62.61 % yield). mp = 166-167.5 °C; UV  $\lambda_{\text{max}}$ : 363.5 nm (DMSO);  $^1\text{H NMR}$  (400 MHz, DMSO):  $\delta_{\text{H}}$  8.56 (d, 1H,  $J = 5.40$  Hz, quinoline-H), 8.02 (d, 1H,  $J = 2.08$  Hz, quinoline-H), 7.85 (d, 1H,  $J = 8.90$  Hz, quinoline-H), 7.43 (dd, 1H,  $J = 8.91, 2.06$  Hz, quinoline-H), 7.04 (d, 1H,  $J = 5.24$  Hz, quinoline-H), 6.98 (d, 1H,  $J = 7.92$  Hz, Ar-H), 6.74 (d, 1H,  $J = 2.24$  Hz, Ar-H), 6.55 (bs, 1H, OH), 6.40 (s, 2H, fluorene-H), 4.14 (s, 2H,  $\text{CH}_2$ ), 4.0 (s, 2H, aromatic C-NH).  $^{13}\text{C NMR}$  (100 MHz, DMSO):  $\delta_{\text{C}}$



**Scheme 3**

159.55, 152.2, 149.5, 150.4, 147.74, 140.5, 134.6, 129.41, 129.2, 127.93, 125.2, 121.54, 119.27, 118.19, 113.17, 110.47, 103.35, 37.17. IR (in KBr disc): 3205, 1608, 1500, 1450, 1375, 1310, 1255, 1159, 1118, 873, 814  $\text{cm}^{-1}$ . MS (m/z): 393.11 (m+) (100), 285 (37), 271 (22).

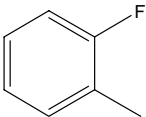
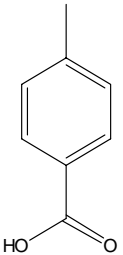
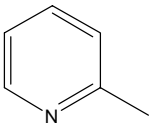
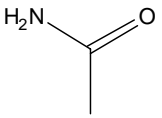
**CS-9: 5-(7-chloroquinolin-4-ylamino)-2-[(4-carboxyphenyl amino) methyl] phenol.** CS-9 was obtained as yellowish brown solid (70.22% yield). mp = 182-184 °C; UV  $\lambda_{\text{max}}$ : 364.0 nm (DMSO);  $^1\text{H}$  NMR (400 MHz, DMSO):  $\delta_{\text{H}}$  8.56 (d, 1H,  $J$  = 5.25 Hz, quinoline-H), 8.04 (d, 1H,  $J$  = 2.06 Hz, quinoline-H), 7.84 (d, 1H,  $J$  = 8.90 Hz, quinoline-H), 7.44 (dd, 1H,  $J$  = 8.91, 2.06 Hz, quinoline-H), 7.02 (d, 1H,  $J$  = 5.23 Hz, quinoline-H), 7.00 (d, 1H,  $J$  = 7.93 Hz, Ar-H), 6.75 (d, 1H,  $J$  = 2.20 Hz, Ar-H), 6.58 (bs, 1H, OH), 3.98 (aromatic C-NH), 6.64 (d, 1H, paraaminobenzoic acid-H), 7.91 (d, 2H,

paraaminobenzoic acid-H).  $^{13}\text{C}$  NMR (100 MHz, DMSO)  $\delta_{\text{C}}$  168.38, 160.44, 152.55, 150.12, 149.21, 146.99, 140.33, 136.57, 129.2, 127.1, 122.4, 119.50, 118.57, 112.53, 110.54, 103.28, 48.28. IR (in KBr disc) 3405, 3200, 1680, 1600, 1505, 1459, 1377, 1336, 1280, 1113, 815, 764  $\text{cm}^{-1}$ . MS (m/z): 491.10 (m+) (100), 285 (37), 271 (22).

**CS-10: 5-(7-chloroquinolin-4-ylamino)-2-[(2-hexylamino) methyl] phenol**

CS-10 was obtained as dark brown solid (67.39% yield); mp = 110-112 °C; UV  $\lambda_{\text{max}}$ : 362.5 nm (DMSO);  $^1\text{H}$  NMR (400 MHz, DMSO):  $\delta_{\text{H}}$  8.53 (d, 1H,  $J$  = 5.20 Hz, quinoline-H), 8.01 (d, 1H,  $J$  = 2.21 Hz, quinoline-H), 7.84 (d, 1H,  $J$  = 8.98 Hz, quinoline-H), 7.43 (dd, 1H,  $J$  = 9.07, 2.20 Hz, quinoline-H), 7.00 (d, 1H,  $J$  = 5.53 Hz, quinoline-H), 6.95 (d, 1H,  $J$  = 7.98 Hz, Ar-H), 6.70 (d, 1H,  $J$  = 2.08 Hz, Ar-H), 6.54 (bs, 1H, OH), 3.75 (s, 2H,  $\text{CH}_2$ ), 1.29 (8H,  $\text{CH}_2\text{CH}_2\text{CH}_2\text{CH}_2$ ), 0.96 (t, 6H,  $J$  = 7.30 Hz,  $\text{CH}_2\text{CH}_3$ ).  $^{13}\text{C}$  NMR (100 MHz, DMSO):  $\delta_{\text{C}}$  159.41, 151.33, 149.76, 147.49, 135.24, 130.57, 129.08, 128.33, 121.22, 118.91, 112.0, 110.7, 101.9, 48.36, 44.6, 29.55, 27.20, 21.55, 14.15. IR (in KBr disc): 3200, 29.30, 2858, 1614, 1575, 1460, 1375, 1327, 1256, 1117, 874, 816, 772  $\text{cm}^{-1}$ . MS (m/z): 383.2 (m+) (100), 285 (30).

**Table 1: Substituents of designed compounds**

Compounds	$\text{R}_1$
CS-8	
CS-9	
CS-10	" $(\text{CH}_2)_5\text{CH}_3$
CS-11	
CS-12	

**CS-11: 5-(7-chloroquinolin-4-ylamino)-2-[(pyridine-2-ylamino) methyl] phenol**

CS-11 was obtained a pale brown solid (69.02% yield); mp = 139-141 °C; UV  $\lambda_{\text{max}}$ : 364.0 nm (DMSO);  $^1\text{H}$  NMR (400 MHz, DMSO):  $\delta_{\text{H}}$  8.55 (d, 1H,  $J$  = 5.40 Hz, quinoline-H), 8.11 (d, 1H, 2-pyridine), 8.01 (d, 1H,  $J$  = 2.09 Hz, quinoline-H), 7.82 (d, 1H,  $J$  = 9.06 Hz, quinoline-H), 7.44 (dd, 1H,  $J$  = 9.06, 2.07 Hz, quinoline-H), 7.00 (d, 1H,  $J$  = 5.41 Hz, quinoline-H), 6.97 (d, 1H,  $J$  = 7.95 Hz, 2.07 Hz, Ar-H), 6.58 (bs, 1H, OH), 6.69 (s, 1H, 2-pyridine), 4.25 (s, 2H,  $\text{CH}_2$ ).  $^{13}\text{C}$  NMR (100 MHz, DMSO):  $\delta_{\text{C}}$  158.7, 152.33, 150.50, 148.22, 147.77, 139.3, 135.55, 131.45, 129.23, 126.55, 121.79, 118.97, 112.0, 110.10, 101.9, 35.0. IR (in KBr disc): 3200, 1699, 1654, 1575, 1450, 1249, 1118, 852, 811, 764  $\text{cm}^{-1}$ . MS (m/z): 375.9 (m+).

**CS-12: 1-[4-(7-Chloroquinolin-4-ylamino)-2-hydroxyphenyl] methyl urea**

CS-12 was obtained as yellowish brown solid (68.99% yield). mp = 169-171 °C; UV  $\lambda_{\text{max}}$ :

363.5 nm (DMSO); <sup>1</sup>H NMR (400 MHz, DMSO):  $\delta_{\text{H}}$  8.57 (d, 1H,  $J = 5.40$  Hz, quinoline-H), 8.03 (d, 1H,  $J = 2.10$  Hz, quinoline-H), 7.84 (d, 1H,  $J = 8.90$  Hz, quinoline-H), 7.45 (dd, 1H,  $J = 5.24$ , quinoline-H), 7.02 (d, 1H,  $J = 7.95$  Hz, Ar-H), 6.76 (d, 1H,  $J = 2.21$  Hz, Ar-H), 6.51 (bs, 1H, OH), 5.98 (s, 3H,

urea). <sup>13</sup>C NMR (100 MHz, DMSO):  $\delta_{\text{C}}$  162.7, 159.29, 152.33, 149.23, 147.2, 141.50, 134.73, 130.11, 128.71, 126.89, 121.55, 119.10, 118.27, 111.22, 107.70, 100.9, 36.0. IR (in KBr disc): 3200, 1690, 1608, 1460, 1377, 1327, 1255, 1119, 816, 754 cm<sup>-1</sup>. MS (m/z): 341.8 (m+).

**Table 2: In vitro antiplasmodial activity of synthesized compounds against chloroquine sensitive RKL-2 strain of Plasmodium falciparum**

Code No. (Compounds)	Concentrations Employed ( $\mu\text{g/ml}$ )	Number of parasites/100 infected RBCs			Percentage Inhibition of Schizont Maturation
		Rings	Trophozoites	Schizonts	
CS-10	100	0	0	0	100
	50	0	0	0	100
	10	0	0	0	100
	2.0	100	0	0	100
	1.0	100	0	0	100
	0.5	100	0	0	100
	0.25	100	0	0	100
	0.125	95	3	2	97.1
	0.063	70	32	8	88.4
CS-9	100	100	0	0	100
	50	100	0	0	100
	10	100	0	0	100
	2.0	100	0	0	100
	1.0	100	0	0	100
	0.5	100	0	0	100
	0.25	36	25	46	48.88
	0.125	19	23	73	18.88
	0.063	0	16	82	8.88
CS-12	0.031	45	61	100	0
	100	100	0	0	100
	50	100	0	0	100
	10	100	14	20	77.77
	2.0	100	0	53	41.11
CS-11	1.0	82	0	89	1.11
	100	0	45	58	35.55
	50	0	29	77	14.44
	10	45	22	88	2.22
CS-8	2	100	0	100	0
	100	100	66	87	3.33
	50	100	91	100	0
Chloroquine	0.125	100	0	0	100
	0.063	100	0	0	100
	0.031	68	8	29	67.67
	0.015	22	25	71	21.11
Control	-	0	10	90	-

### ***In vitro* antiplasmodial evaluation**

All the synthesized compounds were screened for antiplasmodial activity in the Regional Medical Research Centre (Indian Council of Medical Research), N.E. Region, Dibrugarh, Assam, India.

The *in vitro* antiplasmodial bioassay was carried out in 96 well microtitre plates according to the microassay protocol of Rieckmann and co-workers with minor modifications (Rieckmann *et al.*, 1978; Desjardins, 1984). The cultures of *P. falciparum* RKL-2 strain were maintained in medium RPMI 1640 supplemented with 25 mM HEPES, 1% D-glucose, 0.23% sodium bicarbonate and 10% heat inactivated human serum (Trager & Jensen, 1976). The asynchronous parasites of *P. falciparum* were synchronized after 5% D-sorbitol treatment to obtain only the ring stage parasitized cells (Lambros & Vanderberg, 1979). For carrying out the assay, an initial ring stage parasitaemia of 0.8 to 1.5% at 3% haematocrit in a total volume of 200  $\mu$ l of medium RPMI-1640 was determined by Jaswant Singh Bhattacharya (JSB) staining to assess the percent parasitaemia (rings) and uniformly maintained with 50% RBCs (O<sup>+</sup>) (Singh, 1956). A stock solution of 5 mg/ml of each of the test samples was prepared in DMSO and subsequent dilutions were prepared with culture medium. The diluted samples in 20  $\mu$ l volume were added to the test wells so as to obtain final concentrations (at five fold dilutions) ranging between 0.4  $\mu$ g/ml to 100  $\mu$ g/ml in duplicate well containing parasitized cell preparation. The culture plates were incubated at 37°C in a candle jar. After 36 to 40 h incubation, thin blood smears from each well were prepared and stained with JSB stain (Singh, 1956; Panjarathinam, 2007). The slides were microscopically observed to record maturation of ring stage parasites into trophozoites and schizonts in presence of different concentrations of the test agents. The test concentration which inhibited the complete maturation into schizonts was recorded as the minimum inhibitory concentrations (MIC). Chloroquine was used as the reference drug.

### **Results of *in vitro* antiplasmodial evaluation**

The mean number of rings, trophozoites and schizonts were recorded per 100 parasites from duplicate wells after incubation for 38 hours, and

percent maturation inhibition with respect to the control group are summarized in the Table 2. The minimum inhibitory concentration (MIC) values (in Table 2) are indicated in bold italic form.

### **DISCUSSION**

A series of new isoquine analogues i.e. 7-chloro-4-aminoquinoline Mannich base derivatives were synthesized from commercially available starting materials. In this series the 4'-diethylamino function of isoquine is replaced by a 4'-primary amino function. The synthesis involved the preparation of Mannich base by Mannich reaction of the 3-hydroxyacetanilide followed by hydrolysis of the amide function of the Mannich base. The hydrolysis product (Mannich substituted 3-aminophenol) was subsequently coupled with 4, 7-dichloroquinoline to provide the five designed compounds. The compounds were characterized by various spectrometric analysis and the results of which are characteristic of the anticipated structure of the synthesized compounds.

All the synthesized compounds constituted a series with modification at the lateral amino group of the side chain (CS-8 to CS-12). The compounds were evaluated for their *in vitro* antiplasmodial activity against the chloroquine sensitive RKL-2 strain of *P. falciparum*. The *in vitro* antiplasmodial bioassay was carried out by JSB stained slide method. All the tested compounds showed negligible to average percentage of killing the parasites. Two of the synthesized compounds (CS-10 and CS-9) showed comparatively better antiplasmodial activity under the given test conditions with MIC values of 0.063 and 0.5  $\mu$ g/ml respectively. But none of the compounds demonstrated any appreciable activity better than the reference drug, chloroquine.

The antiplasmodial screening result reflects that the compound (CS-10) with alkyl substituted amino group side chain (hexylamine) was found to be the most potent, showing highest antiplasmodial activity (MIC 0.25  $\mu$ g/ml) than the compound (CS-9) containing amino group with aromatic ring (para amino benzoic acid). The compound (CS-12) with urea side chain and the compound (CS-11) with amino group in heterocyclic ring (2-aminopyridine) showed comparatively lower

action. The compound (CS-8) containing amino group in halogen substituted phenyl ring (2-fluorobenzenamine) exhibited negligible and lowest activity. CS-11 and CS-8 did not exhibit MIC under their highest concentration (100 µg/ml) tested against the experimental protozoa. Though none of the synthesized compound (CS-8 to CS-12) demonstrated promising level of antiplasmodial activity as compared to reference chloroquine, however, the compound with aliphatic side chain (CS-10) showed marked level of activity in a concentration dependent manner as compared with the aromatic amines, except CS-9. However, the urea substituted compound showed somewhat lower action.

There are provisions to assess the extent of antiplasmodial activity of synthesized compounds against the other strains and species of *Plasmodium*

and some of these works are presently underway. The novel 7-chloro-4-aminoquinoline derivatives i.e. isoquine analogues, synthesized and evaluated in the present study may be worthwhile for further modification of the isoquine structure in the antimalarial research in pursuit of a newer generation of 4-aminoquinoline antimalarials in future.

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#### REFERENCES

1. Snow R.W., Guerra C.A., Noor A.M., Myint H.Y., Hay S.I. *Nature*, **434**: 214-217 (2005).
2. Anonymous. Conquering, Suffering, and Enriching Humanity: The World Health Report. World Health Organisation Publishers, Geneva, 862 (1997).
3. Olliaro P., Nevill C., Lebras J., Ringwald P., Mussano P., Garner P., Brousseau P. *Lancet*, **348**: 1196-1201 (1996).
4. Lind D.E., Levi J.A., Vincent P.C. *Br. Med. J.*, **1**: 458-460 (1973).
5. Neftel, K.A., Woodtly W., Schmid M. *Br. Med. J.*, **292**: 721- 723 (1986).
6. Harrison A.C., Kitteringham N.R., Clarke J.B., Park B.K. *Pharmacol.*, **43**: 1421-1430 (1992).
7. Maggs J.L., Kitteringham N.R., Park B.K. *Biochem. Pharmacol.*, **37**: 303-311 (1988).
8. Casteel D.A. Antimalarial agents. In: Burger's medicinal chemistry and drug discovery, 6<sup>th</sup> Ed., Vol. V: Chemotherapeutic agents. Abraham DJ Editor. John Wiley & Sons Inc., Hoboken and New Jersey, 920-1031 (2003).
9. O'Neill P.M., Willock D.J., Hawley S.R., Bray P.G., Storr R.C., Ward S.A., Park B.K. *J. Med. Chem.*, **40**: 437-448 (1997).
10. O'Neill P.M., Hawley S.R., Bray P.G., Ward S.A., Park B.K. *Pharmacol. Ther.*, **77**: 29-58 (1998).
11. O'Neill P.M., Mukhtar A., Stocks P.A., Randle L.E., Hindley S., Ward S.A., Storr R.C., Bickley J.F, O'Neil I.A, Maggs J.L, Hughes R.H, Winstanley P.A, Bray P.G, Park B.K. *J. Med. Chem.*, **46**: 4933-4945 (2003).
12. Burckhalter J.H., Tendwick J.H., Jones F.H., Jones P.A., Holcombe W.F., Rawlins A.L. *J. Am. Chem. Soc.*, **70**: 1363-1373 (1948).
13. Rieckmann K.H., Campbell G.H., Sax L.J., Mrema J.E. *Lancet*, **1**: 221-223 (1978).
14. Desjardins R.E. *In vitro* techniques for antimalarial development and evaluation. In: Peters W, Richards WHG editors. Handbook of Experimental Pharmacology, Springer-Verlag, Germany, 179-200 (1984).
15. Trager W., Jensen, J.B. *Science*, **193**: 673-675 (1976).
16. Lambros C., Vanderberg J.P. *J. Parasitol.*, **65**: 418-420 (1979).
17. Singh, J. *Indian J. Malariol.*, **10**: 117-129 (1956).
18. Panjarathinam R. Text Book of Medical Parasitology, 2<sup>nd</sup> Ed., Orient Longman Pvt. Ltd., Chennai, 329-331 (2007).