APPENDIX-I

Progress Report

1.	Project Title	: **Design and development of novel antimalarial agents**
2.	Name of Principal Investigator	: **Prof. M. R. Yadav**
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3.	UGC approval Letter No. and Date	: **F. No.-43-492/2014(SR) dated:- October 2015**
4.	Name of the Research Fellow	: **Ghuge Rahul Baburao**
5.	Effective date of starting the project	: **01-04-2016**

Design and development of novel antimalarial agents

1. INTRODUCTION

Malaria is one of the leading causes of mortality and morbidity among the parasitic diseases. According to the latest report of WHO 2015, malaria is responsible for 214 million new cases and death of 438000 people worldwide wherein children below the age five and pregnant women are considered to be the prime target. It is speculated to cause death of 1300 children every day. The African region contributed the highest (90%) number of all malaria deaths and it is referred as the disease of the poor.[1-3] The main causative agent of malaria is *Plasmodium, a genus* belonging to the phylum protozoa. *Plasmodium* has four main species *i.e. Plasmodium falciparum*, *P. vivax*, *P. malarie* and *P. ovale* which have the potential of inducing malaria disease in humans. Among these species, *P. falciparum* contributed for 90% of malaria cases worldwide and was responsible for the highest number of malaria deaths annually.[1,4,5] The fifth species of *Plasmodium i.e. P. Knowlesi* is responsible for malarial infection in both humans as well as monkeys in certain areas of southeast Asia.[6] The life cycle of *Plasmodium* parasite is complex one and in order to eradicate the disease, every stage should be considered for the treatment of malaria. The life cycle of *Plasmodium* parasite mainly divided into four stages *i.e.* liver stage, blood stage, transmission stage and mosquito stage.[5,7] The present recommended regimen for the treatment of malaria is about two decades old and almost all of the agents suffer from the drawback of parasitic drug resistance.[8] Issues related to drug resistance, rapid onset of action, safety particularly in children and pregnant women and curing malaria in a single dose needed to be addressed for these new molecules. To address this global issue, there is a need to develop novel lead molecules with diverse scaffolds to which the parasite has not been exposed before and helps to overcome the issue related to the drug resistance.[9] In 2015, WHO adopted "Global Technical Strategy for Malaria 2016-2030" for the reduction of malaria incidence speculating to reduce mortality rate by 90%.[10]

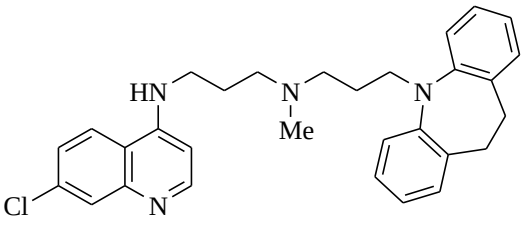
1.1 THERAPEUTIC TARGETS FOR MALARIA[11-13]

The widespread resistance to the all existing antimalarials including frontline agents such as artemisinin, chloroquine and sulfadoxine–pyrimethamine provoked a major challenge for the treatment and eradication of malarial disease. In the current scenario development of novel antimalarial agents with potential activity against the resistant strains of malaria is of

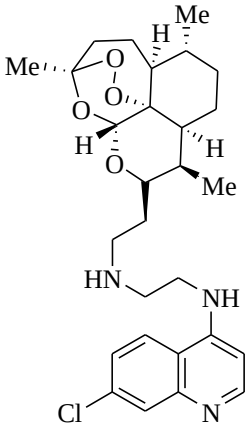
prime importance. Subsequent discovery of novel antimalarial leads that act through specific pathway will be considered on the basis of their location within the malaria parasite. The most important targets on antimalarial parasite mainly include food vacuole, apicoplast, mitochondrial target, cytosolic target, parasite membrane target and cell cycle as a drug target. Amongst these, food vacuole and cytosolic enzymes already established their role as potential targets for the antimalarial drug discovery and also some of the frontline agents such as artemisinin, chloroquine and sulfadoxine–pyrimethamine act on these targets.

2. LITERATURE SURVEY[14-18]

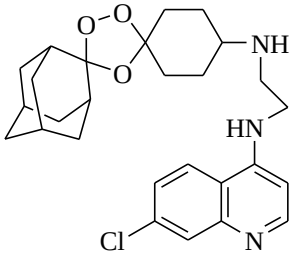
Various researchers throughout the world have been continuously engaged in the development of novel lead molecules active against the malaria strains. Currently, use of molecular hybridization approach is most preferred by scientist for the development of novel lead compounds. Hybrid compounds are constructed by linking two pharmacophore subunits directly or with spacer agents in a single molecule. Some of the representative compounds of this strategy are depicted below.

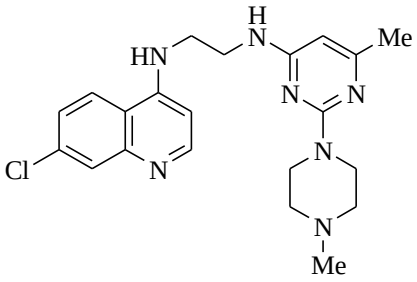


(Burgess *et al. J. Med. Chem*., **2006**)

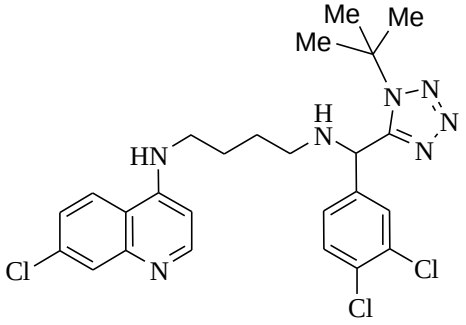


(O'Neill *et al. Bioorg. Med. Chem. Lett.*, **2009**)

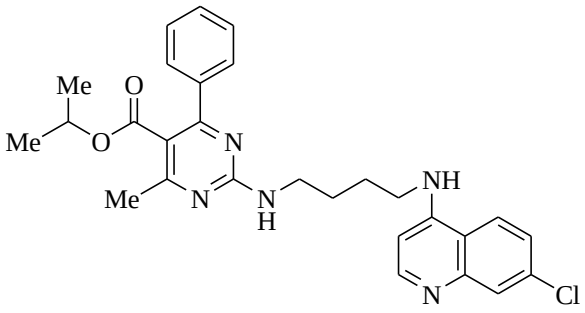




(Manohar *et al. Acs Med. Chem. Lett*. **2012**)



(Tukuluka *et al. Acs Med. Chem. Lett*. **2013**)



(Singh *et al. Eur. J. Med. Chem*., **2013**)

3. RATIONALE OF DESIGN FOR ANTIMALARIAL AGENTS

- Degradation of host red cell hemoglobin within the food vacuole of parasite is the most essential step for the formation of amino acids necessary for the protein synthesis. Degradation of hemoglobin leads to the formation of heam as toxic byproduct and parasite tends to detoxify this heam by converting it into the haemozoin.[19] Hence, the blockade of heam to being converted into the haemozoin is the potential target for the development of antimalarial agents.
- Another crucial target for antimalarial agents is dihydrofolate reductase (DHFR) which played a key step in the conversion of dihydrofolate into tetrahydrofolate. Tetrahydrofolate is essential for the synthesis of purines and thymidylic acid along with certain amino acids which are the important for cell proliferation and cell growth.[19] As DHFR furnish a key step for the formation of nucleic acid precursors it could be regarded as another important target for the synthesis of novel antimalarial lead molecules.
- Keeping in mind, the importance of these two biological targets, we thought logically synthesizing the novel lead molecules containing the two active pharmacophore within the single moiety active against both targets i.e. heam in the food vacuole and DHFR in the cytosol (Fig. 1).
- The molecular hybridization approach proved to be one of the best strategies to overcome drug resistance. The basis of this strategy depends on the hypothesis that during treatment, one of the drugs might be prone to resistance but the other drug has the ability to kill the parasite.

Recently researchers have reported tetrazole containing compounds (**I**; Fig.1) for the management of malaria disease. It was speculated that the tetrazole ring has exceptional affinity for binding heme which ultimately halts the biocrystallization of toxic α-hematin to nontoxic β-hematin. Till date very few reports are available regarding the use of this scaffold for

antimalarial activity and taking into account its crucial role in combating malaria disease we thought of exploring the tetrazole moiety further for the development of novel antimalarial agents effective against both sensitive as well as resistant strains of the parasite.

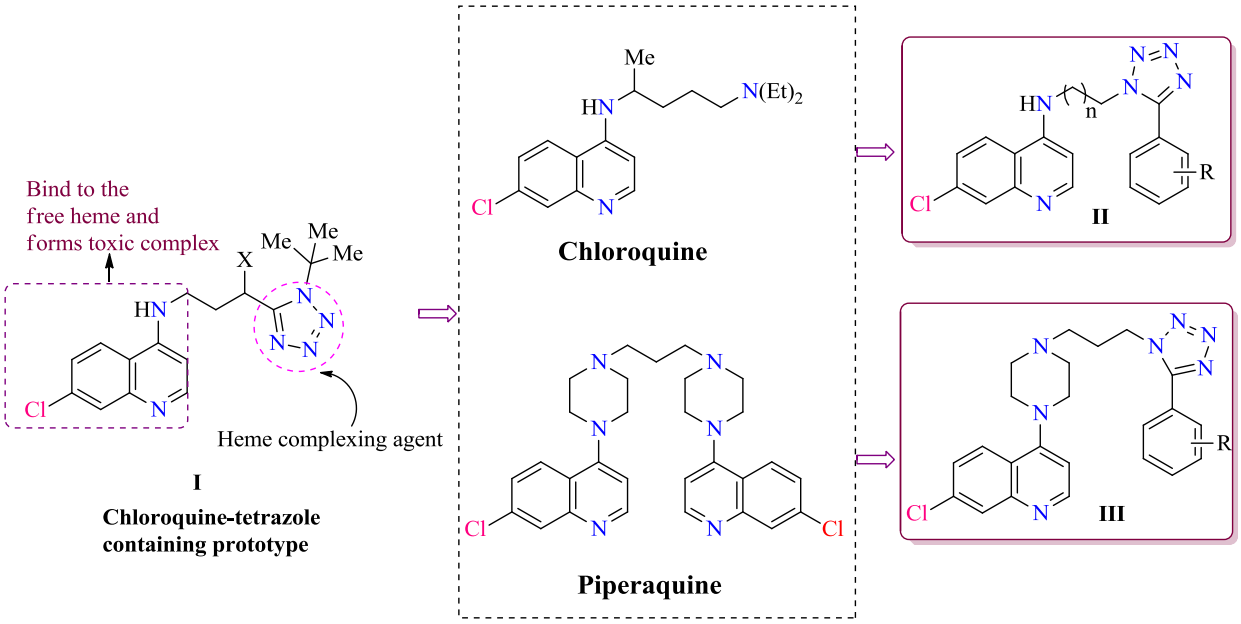


Fig. 1. Designed Hybrid Prototypes (**II** and **III**).

We planned to use two individual active pharmacophores of two marketed drugs (having heme as a target) i.e. 4-amino-7-chloroquinoline core of chloroquine and 7-chloro-4-piperazinoquinoline scaffold of piperaquine for the preparation of novel antimalarial agents. Both of these pharmacophores were separately integrated with the tetrazole scaffold with the help of two or three carbon/piperazinyl linker to offer final hybrid compounds (**II** and **III**; Fig.1).

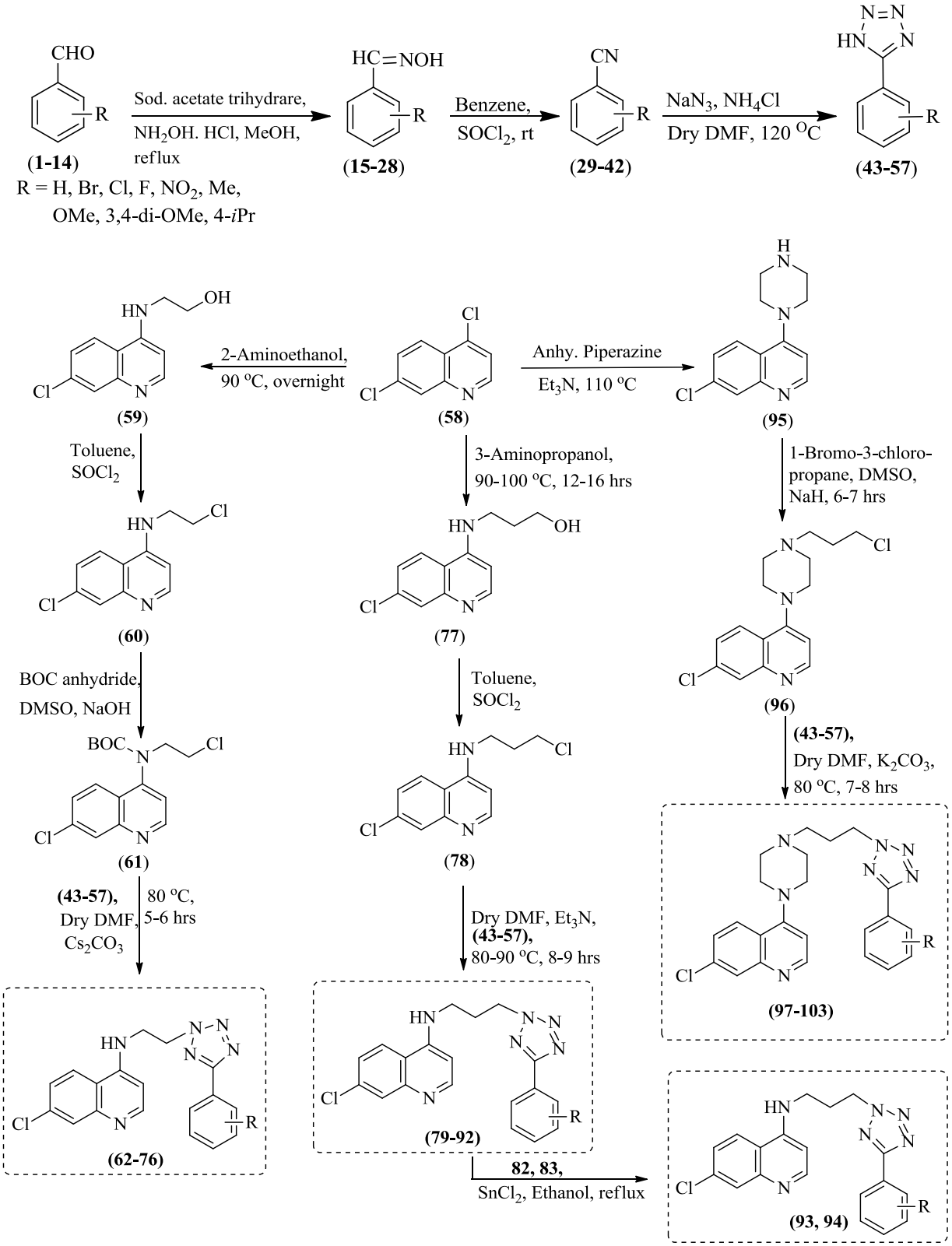
4. AIMS AND OBJECTIVES

- To design novel agents active against both heam in food vacuole and DHFR in cytosol.
- Synthesis of designed prototype (**Fig**. 1) having potential activity against the malarial strains.
- *In vitro* biological evaluation of synthesized compounds.

5. Chemistry

5.1 Synthesis of 7-chloro-4-substituted aminoquinoline derivatives.

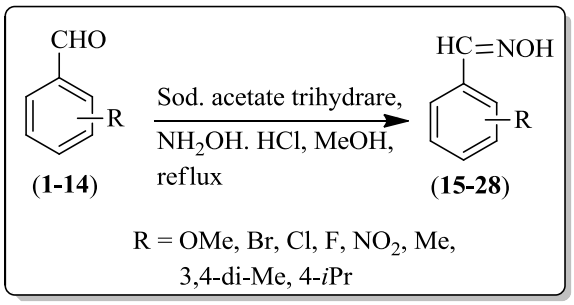
Designed quinoline derivatives were synthesized using the general scheme 1.



Scheme 1: Synthesis of 7-chloro-4-substituted aminoquinoline derivatives.

5.2 Synthesis of substituted aldoximes (15-28)[20-22]

Differently substituted benzaldehydes (**1-14**) were condensed with hydroxylamine hydrochloride and sodium acetate trihydrate in methanol to yield the desired substituted aldoximes (**15-28**, Scheme 1). IR spectra of the compounds **(15-28)** showed characteristic peaks for –OH stretching of oximes in the range of 3200-3400 cm^{-1} and C=N stretching in the range of 1585-1660 cm^{-1} (Table 1).



Scheme 1

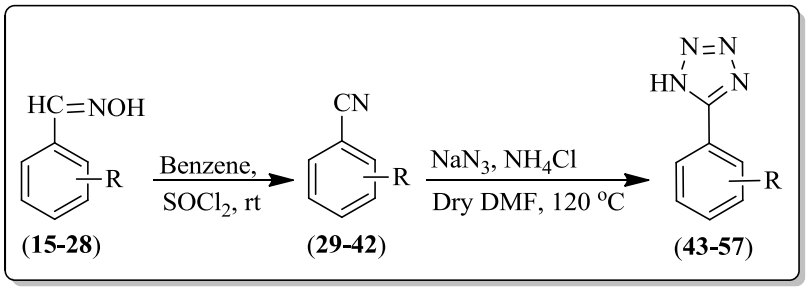
Table 1. Spectral data of the substituted aldoximes (**15-28**)

Comp.	R	IR peaks (cm^{-1})
15	3-OMe	3356 (-OH stretching), 1584 (C=N), 1265 (C-OMe)
16	4-OMe	3300 (-OH stretching), 1607 (C=N), 1251 (C-OMe)
17	3-NO_2	3298 (-OH stretching), 1617 (C=N), 1537 (-NO_2, *asym*.), 1350 (-NO_2, *sym*.)
18	4-NO_2	3308 (-OH stretching), 1604 (C=N), 1537 (-NO_2, *asym*.), 1349 (-NO_2, *sym*.)
19	3-Cl	3195 (-OH stretching), 1629 (C=N)
20	4-Cl	3303 (-OH stretching), 1652 (C=N)
21	2-Br	3286 (-OH stretching), 1589 (C=N)
22	3-Br	3201 (-OH stretching), 1604 (C=N)
23	4-Br	3299 (-OH stretching), 1646 (C=N)
24	3-F	3252 (-OH stretching), 1608 (C=N)
25	4-F	3191 (-OH stretching), 1604 (C=N)
26	4-Me	3279 (-OH stretching), 1605 (C=N)
27	3,4-Di-OMe	3442 (-OH stretching), 1601 (C=N), 1265 (C-OMe)
28	4-*i*Pr	3331 (-OH stretching), 1611 (C=N)

5.3 Synthesis of substituted benzonitriles (29-42)[23-28] **& 5-substituted 1*H*-tetrazoles (43-57)**[29-32]

The obtained aldoximes were dehydrated using thionyl chloride in dry benzene to offer substituted benzonitriles (**29-42**, Scheme 2) exhibiting characteristic signals for nitriles in the range of 2200-2300 cm^{-1} in their IR spectra (Table 2). 5-Substituted 1*H*-tetrazoles (**43-57**, Scheme 2) were obtained through 1,3-dipolar cycloaddition of nitriles (**29-42**) with sodium azide in presence of ammonium chloride in dry DMF. IR spectra of the compounds (**43-57**)

showed disappearance of the nitrile peaks and appearance of characteristic peaks of the tetrazole ring as described in Table 3.



Scheme 2

Table 2: Spectral data of the substituted benzonitriles (**29-42**)

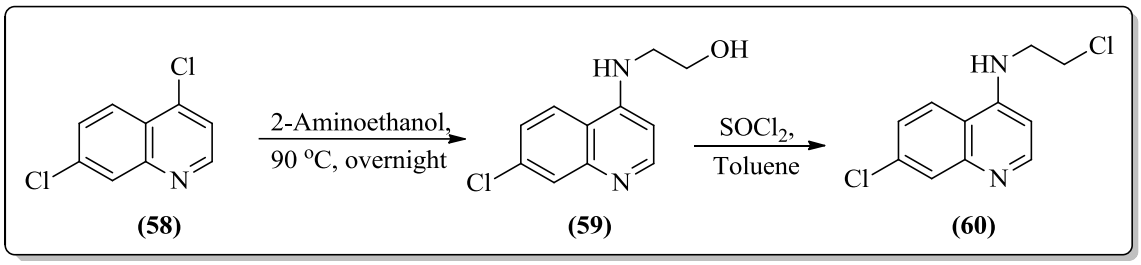
Comp.	R	IR peaks (cm^{-1})
29	3-OMe	2230 (-CN stretching)
30	4-OMe	2215 (-CN stretching), 1258 (-C-OMe)
31	3-NO_2	2234 (-CN stretching), 1528 (-NO_2, *asym*.), 1349 (-NO_2, *sym*.)
32	4-NO_2	2232 (-CN stretching), 1528 (-NO_2, *asym*.), 1349 (-NO_2, *sym*.)
33	3-Cl	2234 (-CN stretching)
34	4-Cl	2222 (-CN stretching)
35	2-Br	2223 (-CN stretching)
36	3-Br	2233 (-CN stretching)
37	4-Br	2252 (-CN stretching)
38	3-F	2236 (-CN stretching)
39	4-F	2232 (-CN stretching)
40	4-Me	2227 (-CN stretching)
41	3,4-Di-OMe	2221 (-CN stretching), 1270 (-C-OMe)
42	4-*i*Pr	2227 (-CN stretching)

Table 3: Spectral data of the 5-substituted 1*H*-tetrazoles (**43-57**)

Comp.	R	IR peaks (cm^{-1})
43	H	3408 (-NH stretching), 1255, 1160, 1055 (tetrazole ring)
44	3-OMe	1250, 1170, 1049 (tetrazole ring)
45	4-OMe	3431 (-NH stretching), 1264, 1182, 1058 (tetrazole ring)
46	3-NO_2	3393 (-NH stretching), 1251, 1160, 1086 (tetrazole ring), 1569 asym, 1349 sym. (-NO_2)
47	4-NO_2	3445 (-NH stretching), 1285, 1133, 1086 (tetrazole ring) 1561 *asym*., 1339 *sym*. (-NO_2),
48	3-Cl	3444 (-NH stretching), 1243, 1157, 1098 (tetrazole ring)
49	4-Cl	3431 (-NH stretching), 1276, 1161, 1095 (tetrazole ring)
50	2-Br	3376 (-NH stretching), 1249, 1165, 1058 (tetrazole ring)
51	3-Br	3461 (-NH stretching), 1244, 1156, 1092 (tetrazole ring)
52	4-Br	3120 (-NH stretching), 1276, 1156, 1075 (tetrazole ring)
53	3-F	3136 (-NH stretching), 1226, 1136, 1081 (tetrazole ring)
54	4-F	3420 (-NH stretching), 1248, 1164, 1052 (tetrazole ring)
55	4-Me	3437 (-NH stretching), 1256, 1162, 1053 (tetrazole ring)
56	3,4-Di-OMe	3420 (-NH stretching), 1274, 1144, 1022 (tetrazole ring)
57	4-*i*Pr	3137 (-NH stretching), 1252, 1160, 1052 (tetrazole ring)

5.4 Synthesis of 2-(7-chloroquinolin-4-ylamino)ethanol (59) and 7-chloro-*N*-(2-chloro ethyl)quinolin-4-amine (60)[33-36]

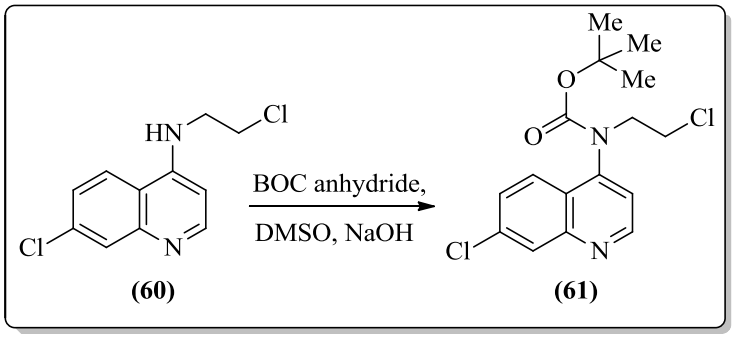
Nucleophilic substitution of 4-chloro group of 4,7-dichloroquinoline (**58**) with 2-aminoethanol offered 2-(7-chloroquinolin-4-ylamino)ethanol (**59**, Scheme 3). IR spectrum of compound **59** showed characteristic signal for free –OH stretching at 3310 cm^{-1} whereas a weak peak was observed at 3110 cm^{-1} for –NH stretching. Compound **59** was chlorinated using thionyl chloride in toluene yielding 7-chloro-*N*-(2-chloroethyl)quinolin-4-amine (**60**, Scheme 3). IR spectrum of compound **60** showed disappearance of OH stretching and the pre-existing signal for –NH stretching was at 3221 cm^{-1}.



Scheme 3

5.5 Synthesis of *tert*.butyl *N*-(-2-chloroethyl)-7-chloroquinolin-4-ylcarbamate (61)

Free –NH functionality of compound **60** was protected with the help of di-*tert*.butyl dicarbonate (BOC anhydride) to avoid intramolecular cyclization (i.e. formation of aziridine ring) to offer the desired compound *tert*.butyl *N*-(2-chloroethyl)-7-chloroquinolin-4-ylcarbamate (**61**, Scheme 4). IR spectrum of compound (**61**) gave C=O stretching of carbamate at 1745 cm^{-1}.

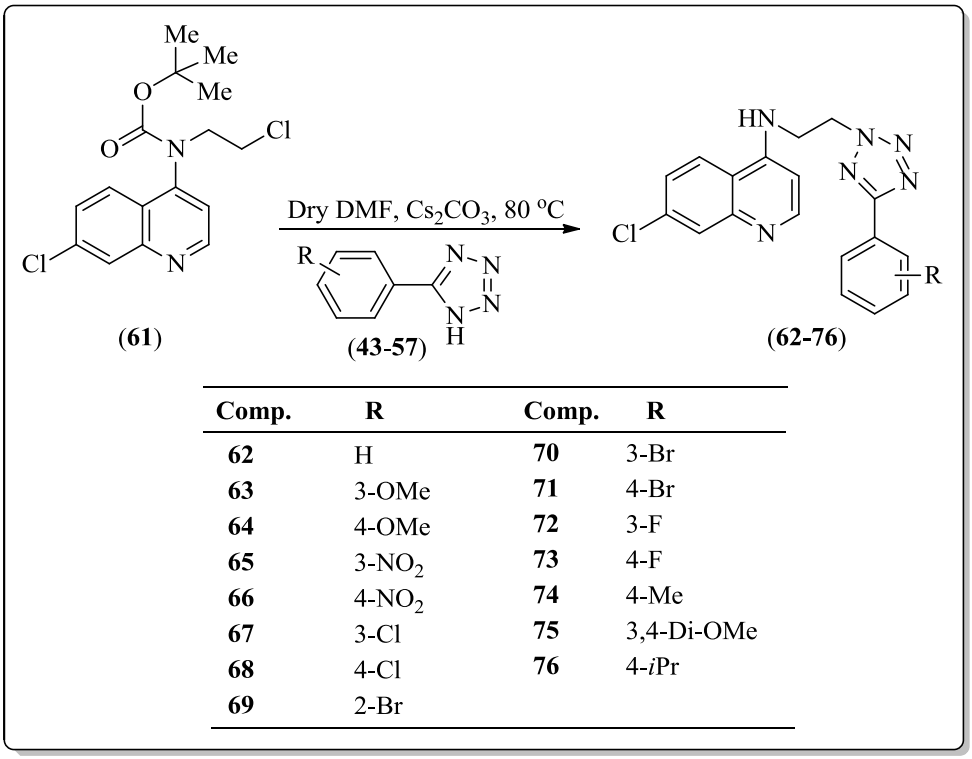


Scheme 4

5.6 Synthesis of 7-chloro-*N*-(2-(5-substituted 1*H*-tetrazol-1-yl)ethyl)quinolin-4-amine derivatives (62-76)

5-Substituted 1*H*-tetrazoles (**43-57**) were reacted with compound (**61**) in the presence of cesium carbonate in dry DMF which offered the desired compounds (**62-76**, Scheme 5). Kazzouli et al.[37] have reported the deprotection of the amine using sodium carbonate in dimethoxyethane (DME) solvent; here we have also obtained BOC. deprotected compounds

using cesium carbonate in DMF solvent. It was evidenced by the absence of C=O stretching of carbamate in IR spectra of the compounds whereas the free –NH stretching appeared in the range of 3200-3300 cm^{-1}.

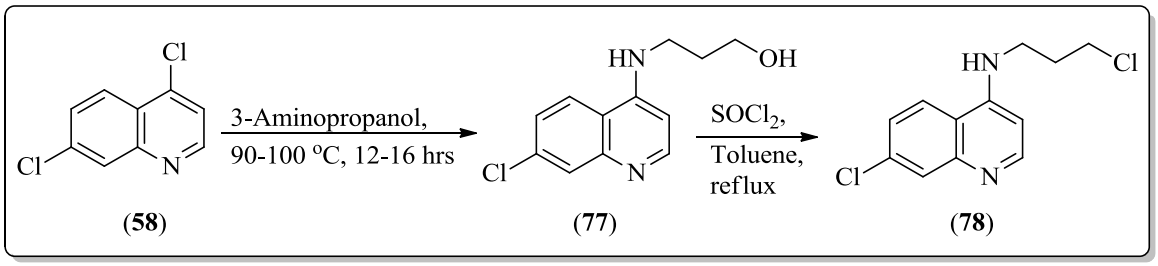


Comp.	R	Comp.	R
62	H	**70**	3-Br
63	3-OMe	**71**	4-Br
64	4-OMe	**72**	3-F
65	3-NO_2	**73**	4-F
66	4-NO_2	**74**	4-Me
67	3-Cl	**75**	3,4-Di-OMe
68	4-Cl	**76**	4-*i*Pr
69	2-Br		

Scheme 5

5.7 Synthesis of 3-(7-chloroquinolin-4-ylamino)propan-1-ol (77) and 7-chloro-*N*-(3-chloro propyl)quinolin-4-amine (78)[33,36]

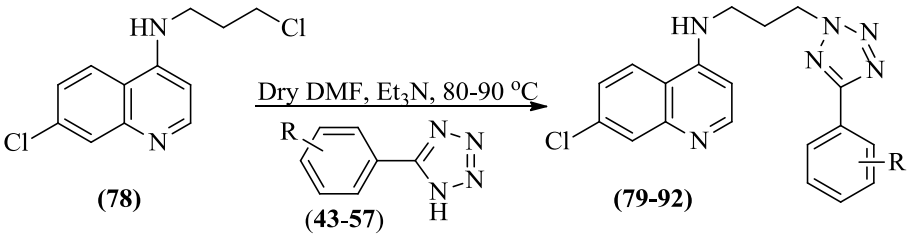
4,7-Dichloroquinoline (**58**) was reacted with 3-amino-1-propanol to offer 3-(7-chloroquinolin-4-ylamino)propan-1-ol (**77**, Scheme 6) which showed peak for free –OH stretching at 3494 cm^{-1} in its IR spectrum. Chlorination of compound (**77**) using thionyl chloride in dry toluene offered 7-chloro-*N*-(3-chloropropyl)quinolin-4-amine (**78**, Scheme 6) in good yield. IR spectrum of compound (**78**) showed –NH stretching at 3210 cm^{-1} and the signal for –OH stretching disappeared completely.



Scheme 6

5.8 Synthesis of 7-chloro-*N*-(3-(5-substituted 1*H*-tetrazol-1-yl)propyl)quinolin-4-amine derivatives (79-92)

5-Substituted 1*H*-tetrazoles (**43-57**) were reacted with compound (**78**) in the presence of triethylamine in dry DMF to offer the desired products (**79-92**, Scheme 7).

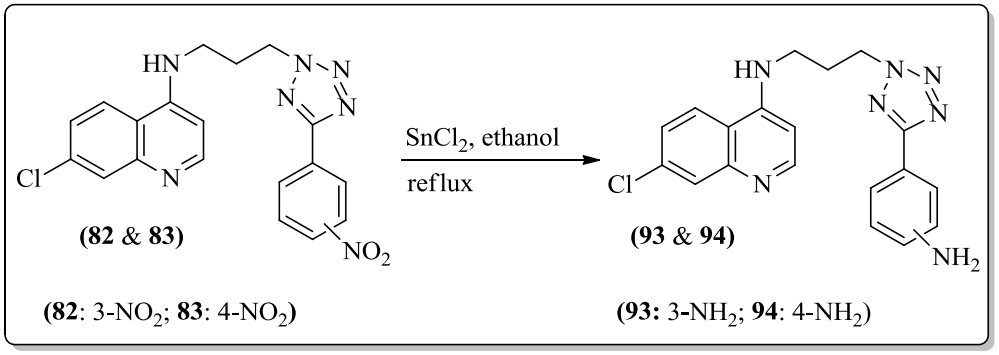


Comp.	R	Comp.	R
79	H	**86**	3-Br
80	3-OMe	**87**	4-Br
81	4-OMe	**88**	3-F
82	3-NO_2	**89**	4-F
83	4-NO_2	**90**	4-Me
84	3-Cl	**91**	3,4-Di-OMe
85	4-Cl	**92**	4-*i*Pr

Scheme 7

5.9 Synthesis of *N*-(3-(5-(3/4-aminosubstituted phenyl)-2*H*-tetrazol-2-yl)propyl)-7-chloroquinolin-4-amine (93 & 94)

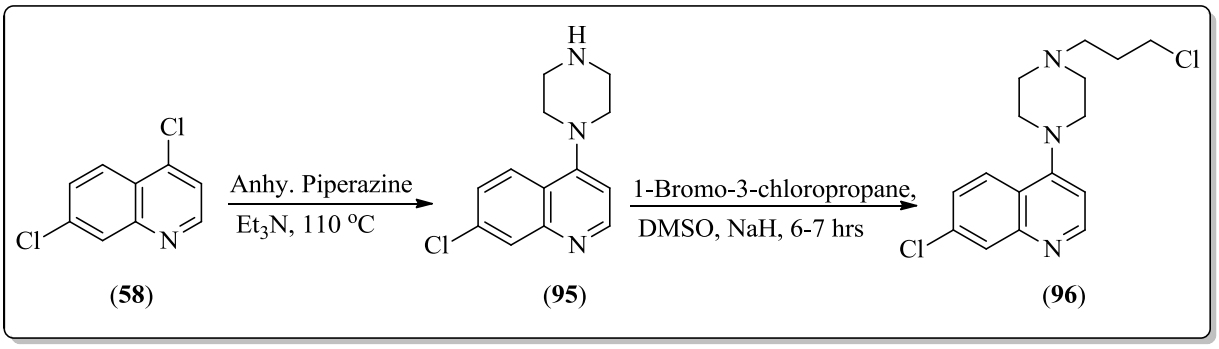
Nitro group of compounds (**82** and **83**) was selectively reduced to amine using stannous chloride in dry ethanol offering compounds *N*-(3-(5-(3-aminophenyl)-2*H*-tetrazol-2-yl)propyl)-7-chloroquinolin-4-amine (**93**) and *N*-(3-(5-(4-aminophenyl)-2*H*-tetrazol-2-yl)propyl)-7-chloroquinolin-4-amine (**94**) respectively (Scheme 8). In IR spectra of both the compounds (**93** and **94**), peaks for nitro group got disappeared and two characteristic signals for the primary amine appeared in the range of 3274 to 3425 cm^{-1}.



Scheme 8

5.10 Synthesis of 7-chloro-4-(piperazin-1-yl)quinoline (95) and 7-chloro-4-(4-(3-chloro propyl)piperazin-1-yl)quinoline (96)[33-36]

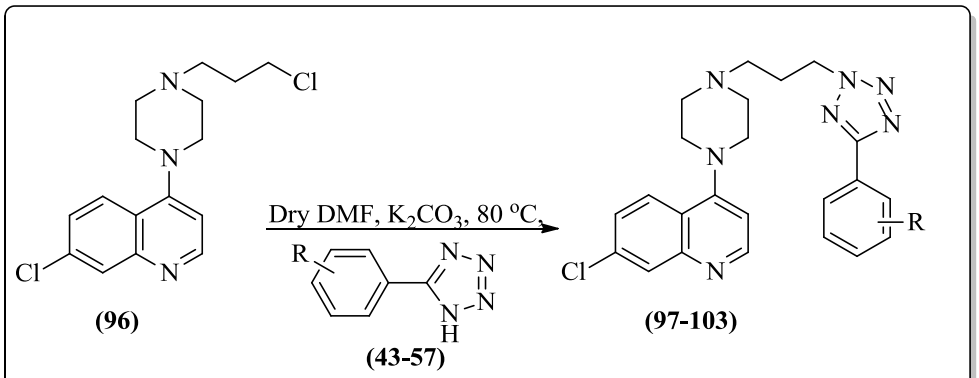
4,7-Dichloroquinoline (**58**) was reacted with anhydrous piperazine in a neat reaction in presence of triethylamine, which offered the desired intermediate 7-chloro-4-(piperazin-1-yl)quinoline (**95**, Scheme 9). IR spectrum of compound (**95**) exhibited a peak for –NH stretching at 3254 cm^{-1}. Alkylation of compound (**95**) was carried out using 2-bromo-3-chloropropane in the presence of sodium hydride in DMSO at room temp. offering the desired 7-chloro-4-(4-(3-chloropropyl)piperazin-1-yl)quinoline (**96**, Scheme 9). In IR spectrum of compound (**96**), signal for the -NH stretching got disappeared.



Scheme 9

5.11 Synthesis of 7-chloro-4-(4-(3-(5-substituted 2*H*-tetrazol-2-yl)propyl)piperazin-1-yl) quinolines (97-103)

5-Substituted 1*H*-tetrazoles were reacted with compound (**96**) in the presence of potassium carbonate in dry DMF offering the desired 7-chloro-4-(4-(3-(5-substituted 2*H*-tetrazol-2-yl)propyl)-piperazin-1-yl)quinoline derivatives (**97-103**, Scheme 10).



Comp.	R	Comp.	R
97	H	**101**	4-Me
98	4-NO_2	**102**	3,4-Di-OMe
99	4-Br	**103**	4-*i*Pr
100	4-F		

Scheme 10

6. Biological

Preliminary biological screening of the synthesized compounds was carried out using SYBR Green I assay against CQ resistant K1 strain at University of Salford, Manchester, UK. All the tested compounds (**62-76** and **79-94**) showed potential for antimalarial activity and reduced parasitaemia load in between 70-15.47 % at 5 µM conc. (Table 1). Determination of IC_{50} values, heme binding assay and cytotoxicity studies of the compounds (**62-76** and **79-94**) is currently in pipeline.

Table 1: *In vitro* biological screening results for compounds (**62-76** and **79-94**).

Comp.	% Parasitaemia	Comp.	% Parasitaemia	Comp.	% Parasitaemia
62	34.65	**73**	20.91	**86**	19.14
63	21.01	**74**	20.41	**87**	102.88
64	19.95	**75**	70.24	**88**	20.21
65	41.15	**76**	21.16	**89**	20.63
66	35.75	**79**	21.76	**90**	19.72
67	20.73	**80**	21.13	**91**	23.02
68	21.45	**81**	23.57	**92**	15.47
69	20.20	**82**	38.62	**93**	25.31
70	20.82	**83**	25.34	**94**	17.91
71	20.59	**84**	19.43	**Control**	100
72	21.26	**85**	19.50		

Compound **97** (**195 in the Fig. 2**) and some of the compounds from other series i.e. (**211-237**, **241-275** and **278**) were evaluated for their antiparasitic activity against CQ resistant INDO strain (Fig. 2) at International Center for Genetic Engineering and Biotechnology (ICGEB), New Delhi, India. Among these compounds, a quinoline derivative (**97**) showed

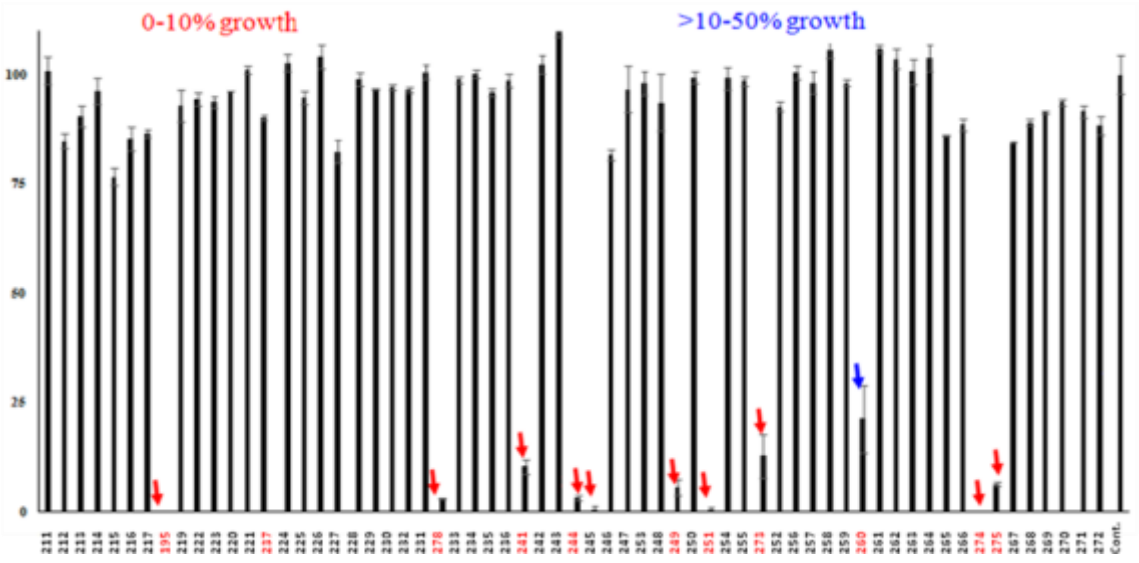
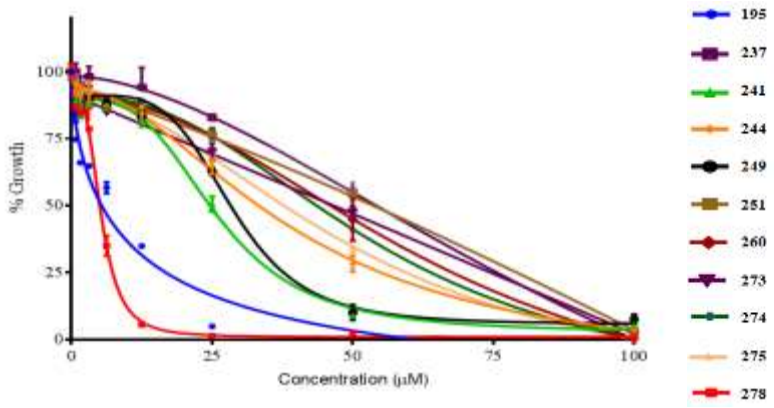
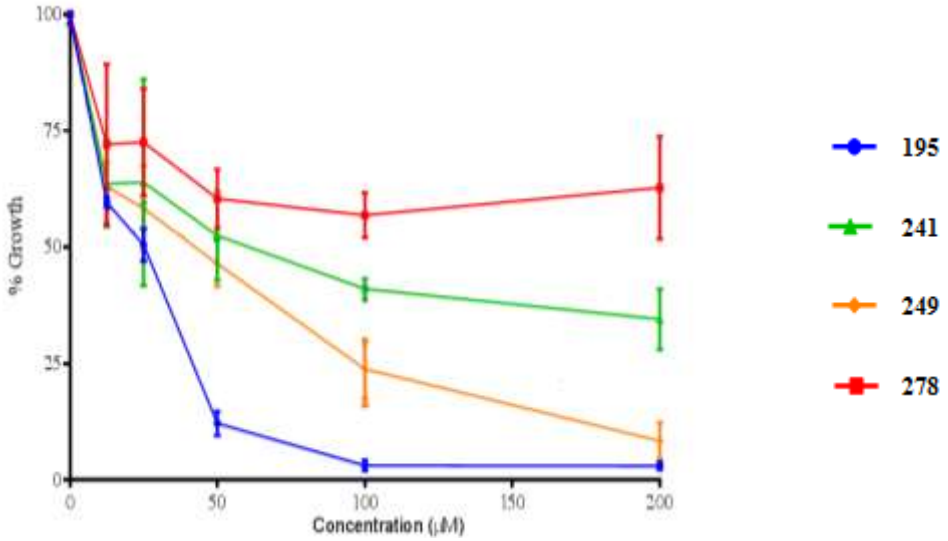


Fig. 2: Screening of % inhibition of *Pf*INDO strain by test compounds (SYBR green assay).



Comp.	195 (97)	237	241	244	249	251	260	273	274	275	278
IC_{50} (µM)	11.30	49.53	26.00	39.90	28.75	43.24	66.20	35.66	55.48	51.78	5.31

Fig. 3: % Growth of *P. falciparum* INDO strain in presence of test compounds.



Comp.	195 (97)	241	249	278
CC_{50} (µM) HUH-7 hepatic cell	19.11	55.50	31.04	> 200
IC_{50} (µM) *Pf*INDO strain	11.30	26.0	28.75	5.30
Selectivity index (CC_{50}/ IC_{50})	1.69	2.13	1.07	> 37.66

Fig. 4: Cell cytotoxicity assay of some promising compounds with HUH-7 cell line.

promising antimalarial activity with an IC_{50} value of 11.30 µM (Fig. 3) and in cytotoxicity study (Fig. 4) it proved to be nontoxic against HUH-7 (hepatic cell) cell line upto conc. of 19.11 µM. Compound **(97)** also exhibited a selectivity index of 1.69. In order to gain

mechanistic details of compound (**97**), its stage specific antimalarial potential was further evaluated on the different stages of life cycle of malaria parasite i.e. on schizont/merozoites, trophozoite and schizont stages. Schizont/merozoite stages of parasite were found to be sensitive to compound (**97**) and inhibited the growth of schizont (36 to 38 h pi) following the 12 h treatment at concentrations ranging from 25 to 100 µM (Fig. 5). The results were acquired from FL-1 channel using flow cytometry. Compound (**97**) inhibited growths of early trophozoites (20 to 24 h pi) stage following 8 h treatment and the results revealed that trophozoites were affected at 25 to 100 µM concentrations (Fig. 6).

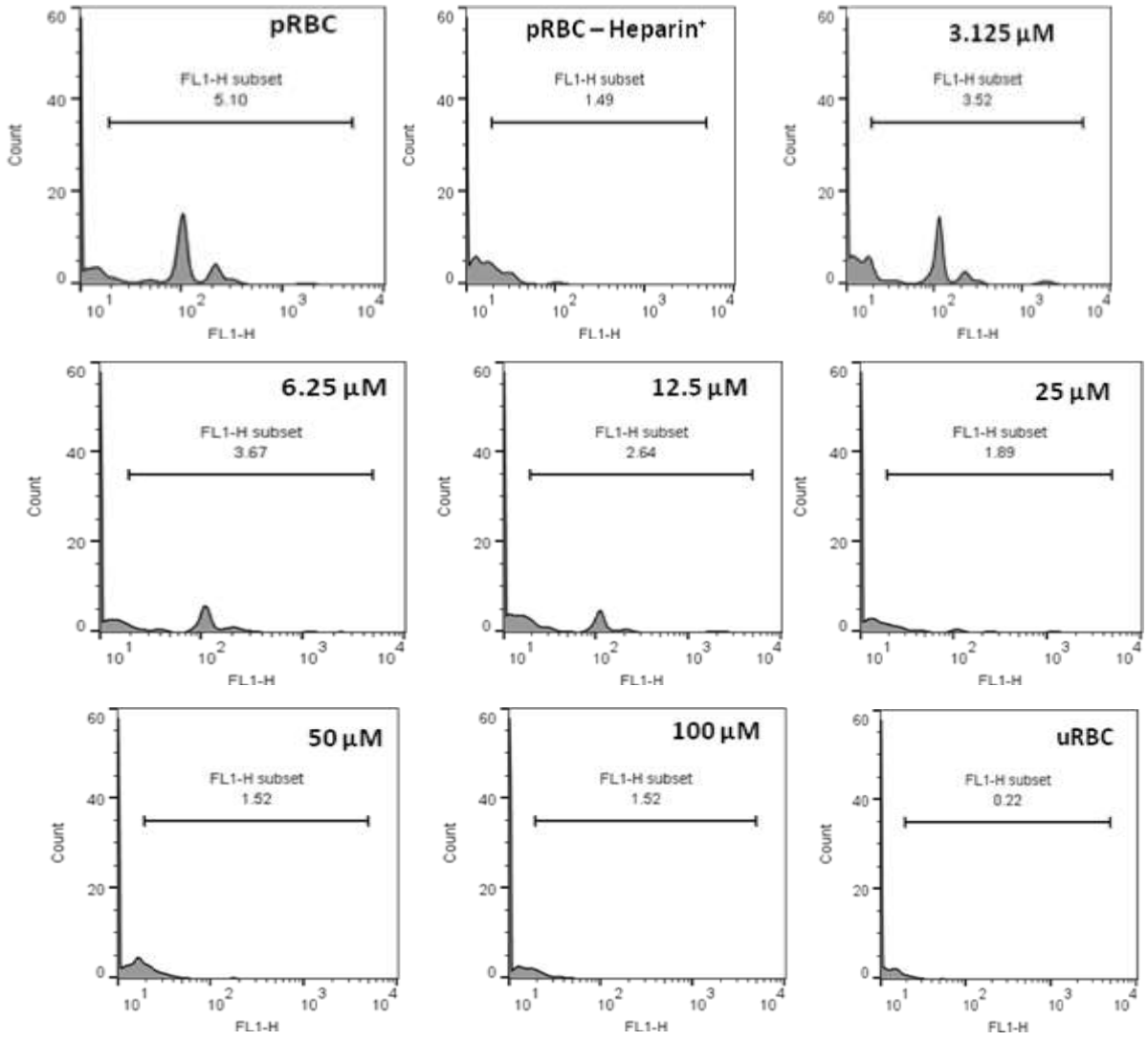
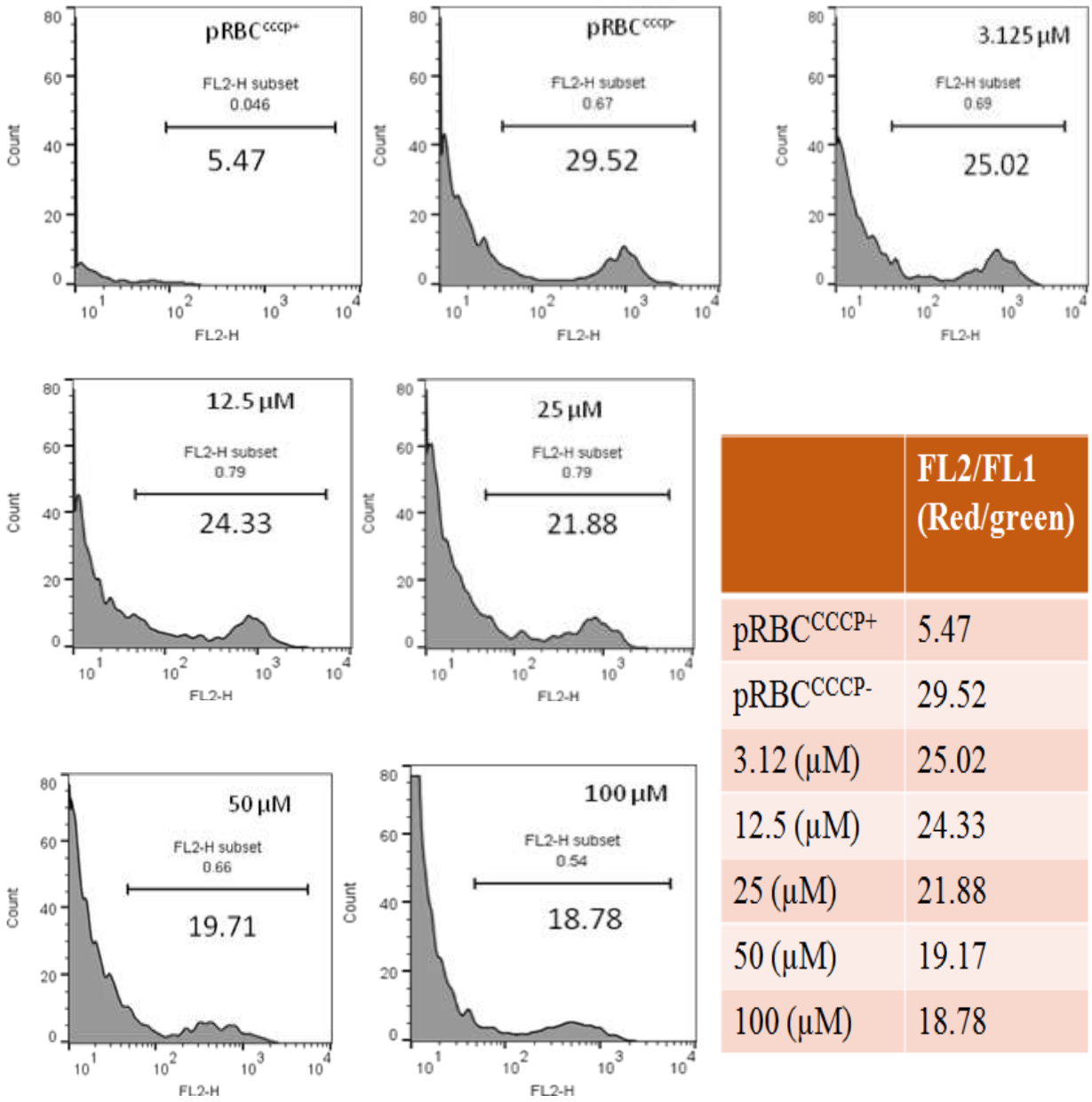


Fig. 5: Assay to confirm inhibitory activity of compound (**97**) against schizonts/merozoites. Heparin is used as positive control (known inhibitor of invasion). Data was acquired from FL-1 channel using flow cytometry.

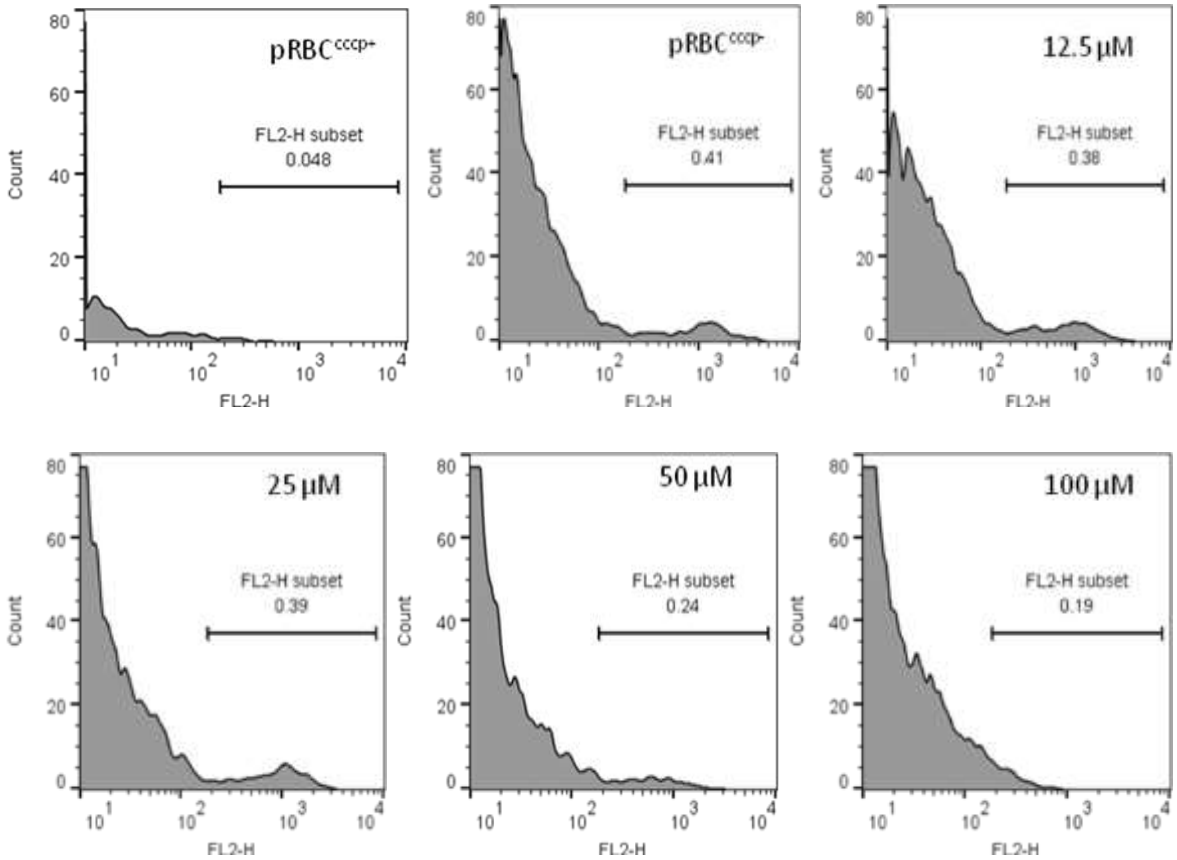


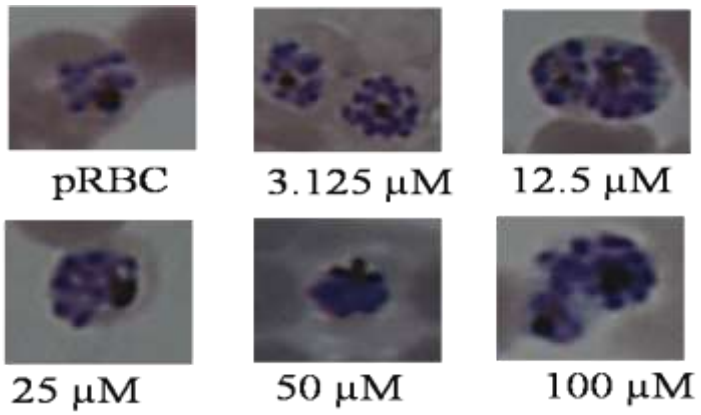
	FL2/FL1 (Red/green)
pRBC^{CCCP+}	5.47
pRBC^{CCCP-}	29.52
3.12 (µM)	25.02
12.5 (µM)	24.33
25 (µM)	21.88
50 (µM)	19.17
100 (µM)	18.78

Fig. 6: Assay to probe effect of compound (**97**) on trophozoites of *Pf*INDO. Cell death was assayed by using JC-1 dye and CCCP was used as control. Histograms were obtained from FL-2 channel in flow cytometry.

Early schizont stage cidal effect of compound (**97**) was tested on schizonts (36-38 h pi) with varying concentrations following 8 h treatment. Results of the experiments were acquired from FL-2 channel of flow cytometry (Fig.7) and the final conclusion is made by comparing the results obtained from the two individual FL-1 and FL-2 channels. Compound (**97**) showed schizontocidal activity at 100 µM concentration as shown in Fig. 7. In order to further test potential of compound (**97**), it was treated with mature schizont (40 to 42 h pi) and the results were obtained from FL-2 channel of flow cytometry (Fig. 8). Comparison of data from both

FL-1 and FL-2 channels revealed that mature schizonts (40 to 42 h pi) were not affected by compound (**97**) even at 100 µM concentration (Fig. 8).

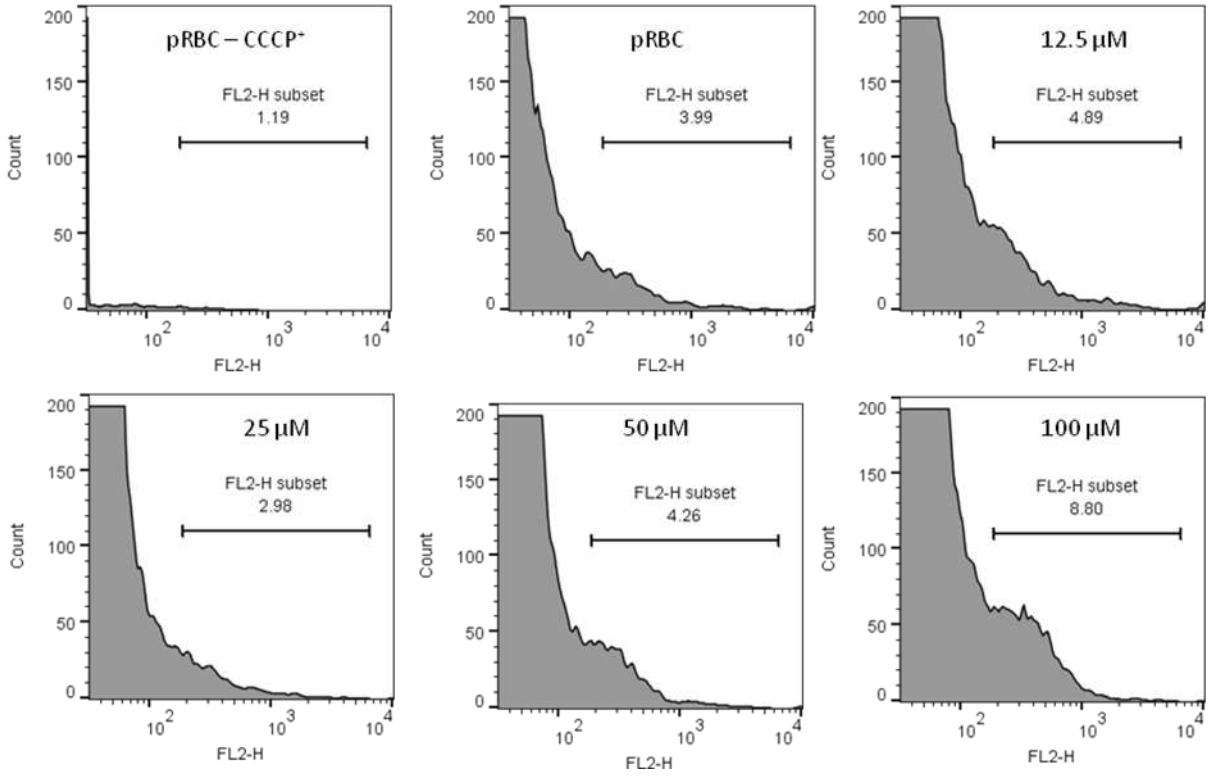




	FL2/FL1 (Red/green)
pRBC^{CCCP+}	2.95
pRBC^{CCCP-}	16.55
12.5 (µM)	16.36
25 (µM)	19.04
50 (µM)	13.49
100 (µM)	8.95

Fig. 7: Assay to probe schizonts (36 to 38 h pi) killing by compound (**97**). Cell count was assayed by using JC-1 dye and CCCP is used as control. Histograms were obtained from FL-2 channel in flow cytometry. Effect of varying concentrations of compound (**97**) concentrations on schizonts was also measured using Giemsa staining.

To probe inhibitor potential of the parasite with mature schizonts (40 to 42 h pi) were treated with compound (**97**) (12 h treatment) with varying concentrations ranging from 1.56 to 100 µM (Fig.9). The results of histogram obtained using flow cytometry and from Giemsa staining (Fig. 9) concluded that compound (**97**) inhibited parasite invasion at 50 and 100 µM. Further, heme binding assay for compound (**97**) is currently under study.



	pRBC^{CCCP+}	pRBC^{CCCP-}	12.5 (µM)	25 (µM)	50 (µM)	100 (µM)
FL2/FL1 (Red/green)	1.02	2.50	2.80	2.19	2.23	2.43

Fig. 8: Assay to probe inhibitory activity of compound (**97**) against mature schizonts (40-42 h pi). Data was acquired from FL-2 channel using flow cytometry.

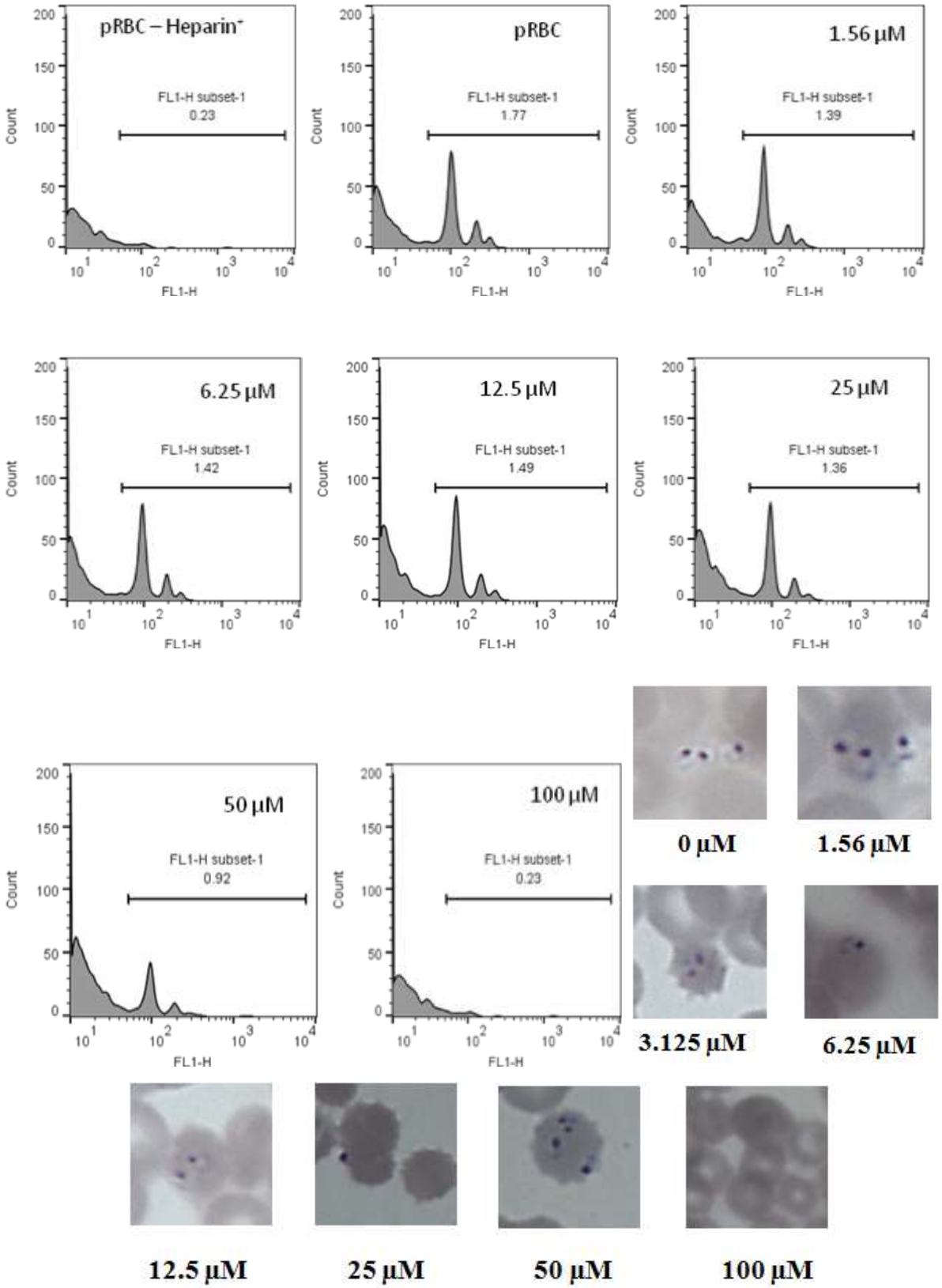


Fig. 9: Assay to probe invasion inhibition of mature schizont (42 to 45 h pi) by compound (**97**) in *Pf*INDO strain. Histograms were obtained from FL-1 channel in flow cytometry.

7. Conclusion

Current work discloses the synthesis and antiplasmodial activity of novel heterocyclic compounds. Synthesis of the compounds was done by adopting three General synthetic schemes. In General scheme-1, 7-chloro-4-substitutedquinolines were clubbed with substituted tetrazoles through varying linkers i.e. ethyl (**62-76**), propyl (**79-92**) and piperazinyl (**97-103**). Among the series of compounds majority of the compounds (**62-74**, **76-86** and **88-94**) exhibited promising antimalarial activity in their preliminary biological screening (SYBR Green assay) and inhibited parasitaemia load (*Pf*K1 strain) to a greater extent (upto 15.47 % of parasitaemia). One of the compounds (**97**), in which quinoline and tetrazole scaffolds were integrated through piperazinyl linker, showed the most promising activity against the *Pf*INDO strain with an IC_{50} value of 11.3 µM. The hybrid (**97**) proved to be nontoxic and the tolerated dose was found to be upto 19.11 µM (CC_{50}) in cell cytotoxicity assay and it also showed selectivity index of 1.69. In stage specific mechanistic study, compound (**97**) showed inhibitory activity against the schizonts/merozoites (36 -38 h pi) at concentrations of 25 to 100 µM. It also affected trophozoites (20 -24 h pi) and showed significant inhibitory activity at a dose of 25 to 100 µM dose. Compound (**97**) showed schizonticidal activity against the schizonts (36 -38 h pi) at a dose of 100 µM but unfortunately it failed to show activity against the mature schizonts (40-42 h pi) even at 100 µM concentration. Ability of the compound (**97**) to invasion inhibition was also evaluated against mature schizonts (40-42 h pi) where it showed its potential to inhibit parasite invasion at 50 and 100 µM doses.

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