Summary

of

UGC MRP

1.	Project Title	: **Design and development of novel antimalarial agents**
2.	Name of Principal Investigator	: **Prof. M. R. Yadav**
	University/College	**Faculty of Pharmacy, The Maharaja Sayajirao University of Baroda, Vadodara, Gujarat.**
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**

SUMMARY

Malaria is one of the deadliest forms of parasitic diseases, having savage magnitude on people's health and countries' socioeconomic outgrowth. It is caused by the parasite of genus *Plasmodium* and in humans the disease is transferred through the bites of infected female anopheles mosquito. Despite continuous efforts to confront the onset of infection, mortality rates and to eradicate malaria, it still ranked among the topmost widespread life-threatening diseases in the world. According to the World Health Organization (WHO) 2018

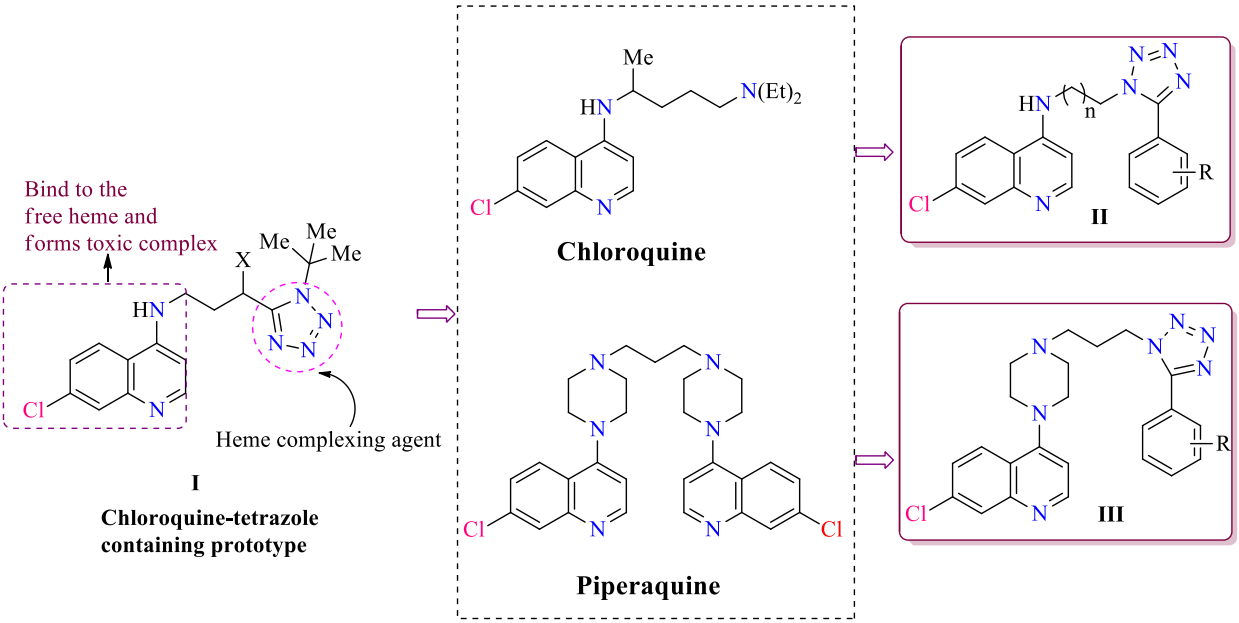


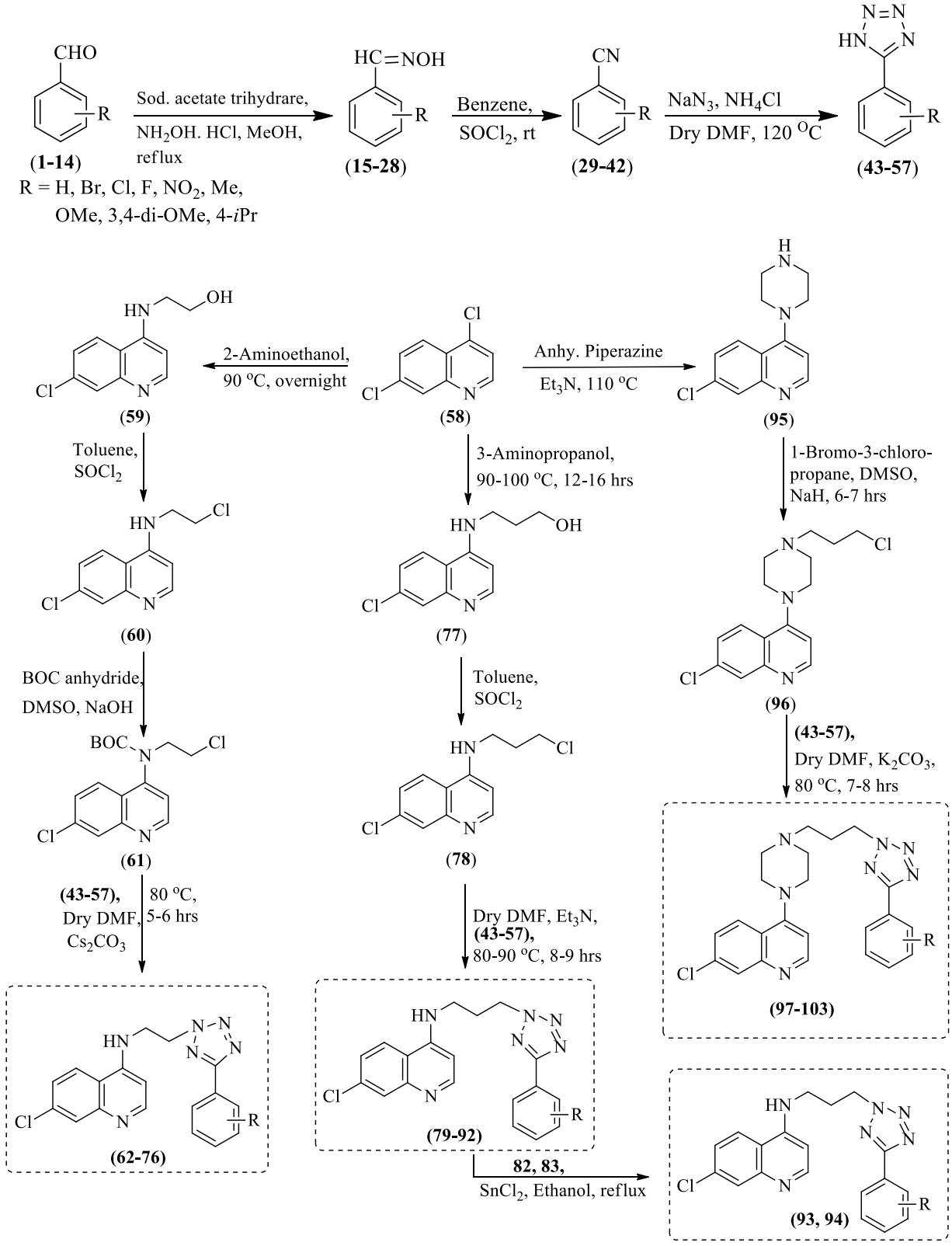
Fig. 1: Rationale of design for the synthesis of proposed hybrid molecules.

world malaria report, an approximate 219 million cases were reported in 2017 with speculated 4,35,000 deaths globally. Children below the age of 5 years and pregnant women are the most affected ones.

Malaria parasite is enriched with diverse number of targets and among these; detoxification cascade of heme by the parasite remains a live target. Importance of this target is also well evidenced, as majority of the antimalarial drugs available in the market have heme as a prime target. Although majority of the drug candidates acting on this target suffer from the drug resistant phenomenon, it is noteworthy to mention that drug resistance does not occur due the altered target (heme) but due to other resistance mechanisms.

Recently researchers have reported that tetrazole ring has an exceptional affinity for binding to the heme which ultimately halts the bio-crystallization of toxic α-hematin to nontoxic β-hematin form. Hence we thought logically to design novel hybrid compounds where diversified heterocyclic scaffolds are individually integrated with tetrazole moiety within a single molecule (hybrid molecules, **Fig.1**) which would be having activity against the detoxification cascade of heme as well as other resistance mechanism related to this target.

To synthesize the proposed compounds of our interest, **General schemes 1** was adopted as depicted.



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

BIOLOGICAL SCREENING

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

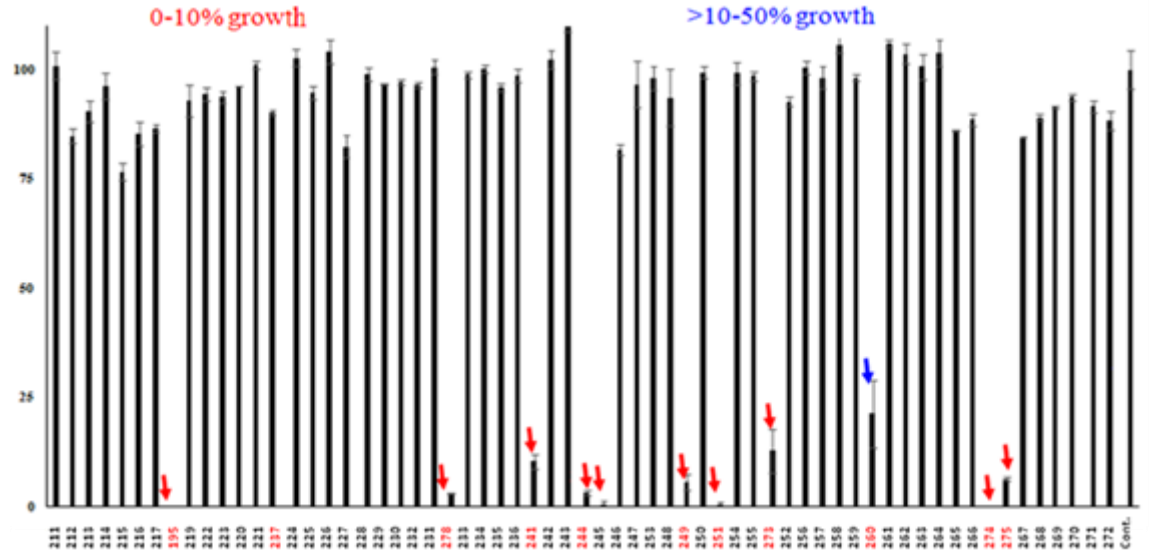
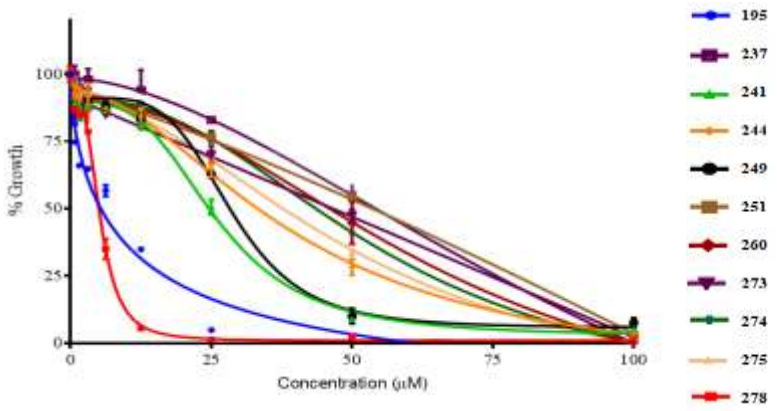
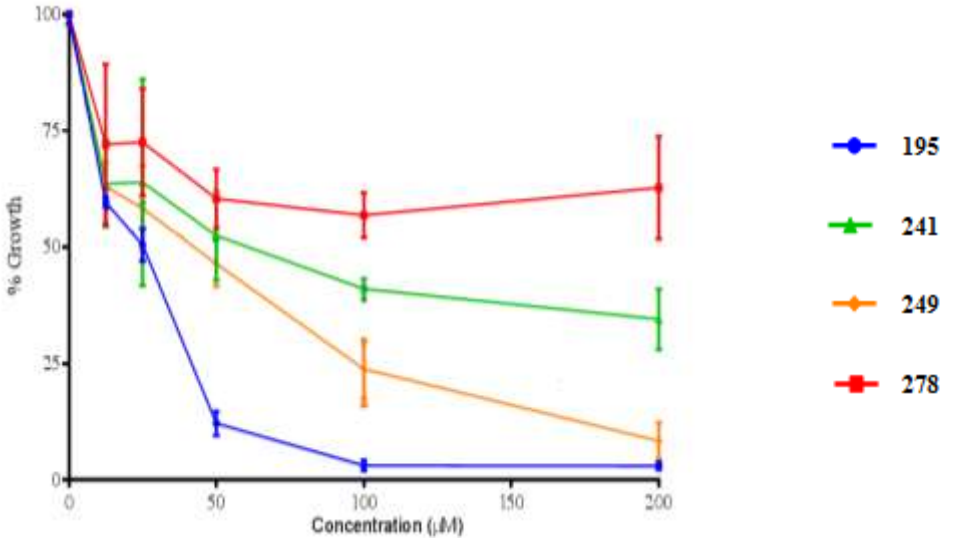


Fig. 2: Screening of % inhibition of *Pf*INDO strain by test compounds (SYBR green assay).



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 IC50 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.

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.