The 8th International Electronic Conference on Medicinal Chemistry (ECMC 2022)
01–30 NOVEMBER 2022 | ONLINE
Chaired by DR. ALFREDO BERZAL-HERRANZ;
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Targeting human β2 adrenergic receptors (*ADRB2*) for modulating blood-stage infection of *Plasmodium falciparum* malaria

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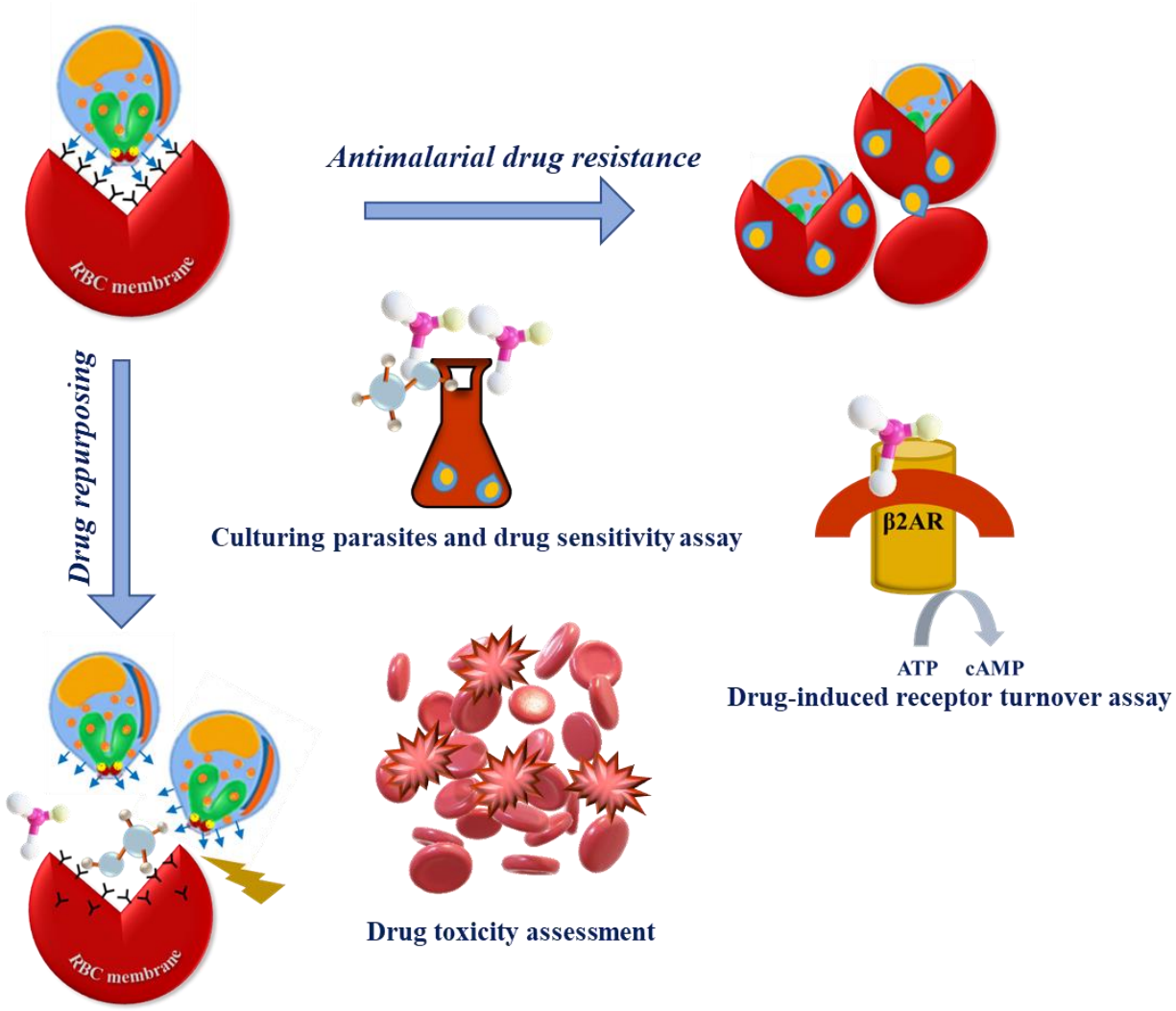
Targeting human β2 adrenergic receptors (*ADRB2*) for modulating blood-stage infection of *Plasmodium falciparum* malaria

Graphical Abstract



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Abstract

Strategies for malarial control are met with difficulties due to insufficiency of control measures and emerging parasite drug resistance. Erythrocytes, mainly their lipid rafts-protein, have been demonstrated as targets of *Plasmodium* parasites interaction for invasion of cells causing infection. GPCRs, *viz.* ADRB2 with their central role in physiological processes and intricate signaling pathways have been the targets for a range of diseases. *In vitro* studies have shown signaling *via* erythrocytic ADRB2 regulates the entry of *P. falciparum*. Prior studies in our laboratory demonstrated genetic variations of membrane proteins; *ADRB2*, *ADORA2A*, *ADORA2B*, *ABCB1* were associated with malaria progression. ADRB2 protein has been extensively studied in the context of etiology and therapeutics of asthma, and its agonists are being used as reliever drugs. Since anti-asthmatics work by internalizing the receptors, well-established drugs with known pharmacokinetic properties and efficacies can be good candidates to treat malaria. Demonstration of proof-of-concept of ADRB2-mediated mechanisms of clinical relevance may provide avenues for drug-repurposing. Erythrocyte cultures were employed to test the anti-malarial activity of β2AR-agonists and antagonists in combination with antimalarials on *P. falciparum*-infected erythrocytes. SYBR green-I fluorescence assay was performed to study parasite inhibition, and the results analyzed for their synergistic action. Agonist-induced receptor desensitization was assayed using flow cytometry and visualized. *In vitro* activities of salbutamol, propranolol, and combination treatments demonstrated parasite inhibition with acceptable haemotoxicity. The receptor internalization was found to be incremental and microscopic observations validating β2AR-desensitization were obtained, providing further mechanistic insights into β2AR-mediated parasite invasion.

Keywords: β2 adrenergic receptor; drug repurposing; invasion; malaria; *Plasmodium*

INTRODUCTION

MALARIA

- Malaria is a vector borne infectious disease caused by *Plasmodium* parasites
- Female *Anopheles* mosquito acts as vectors, humans get infected via mosquito bites
- Mostly seen in tropic and sub tropic regions

PREGNANT WOMEN

are at HIGH RISK of dying from complications of severe malaria

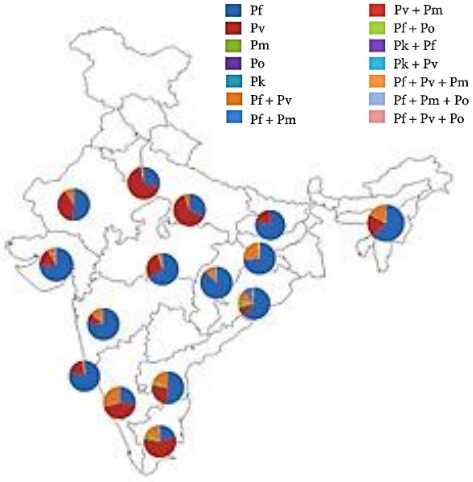
87

Countries and territories had ongoing malaria transmission in 2020.

WHO: World malaria report-241 million cases and 6,27,000 deaths in 2020

3.4 billion

People are at risk of malaria worldwide



Mono-malaria parasites or mixed infection in different states of India

10,000

Travelers are reported to become ill with malaria after returning home (Each year)

A child dies from malaria in Africa

Singh US et al., 2017; WHO Report, 2021

LIFE CYCLE

- **Malaria parasites**
  - *P. falciparum*
  - *P. vivax*
  - *P. knowlesi*
  - *P. ovale*
  - *P. malariae*

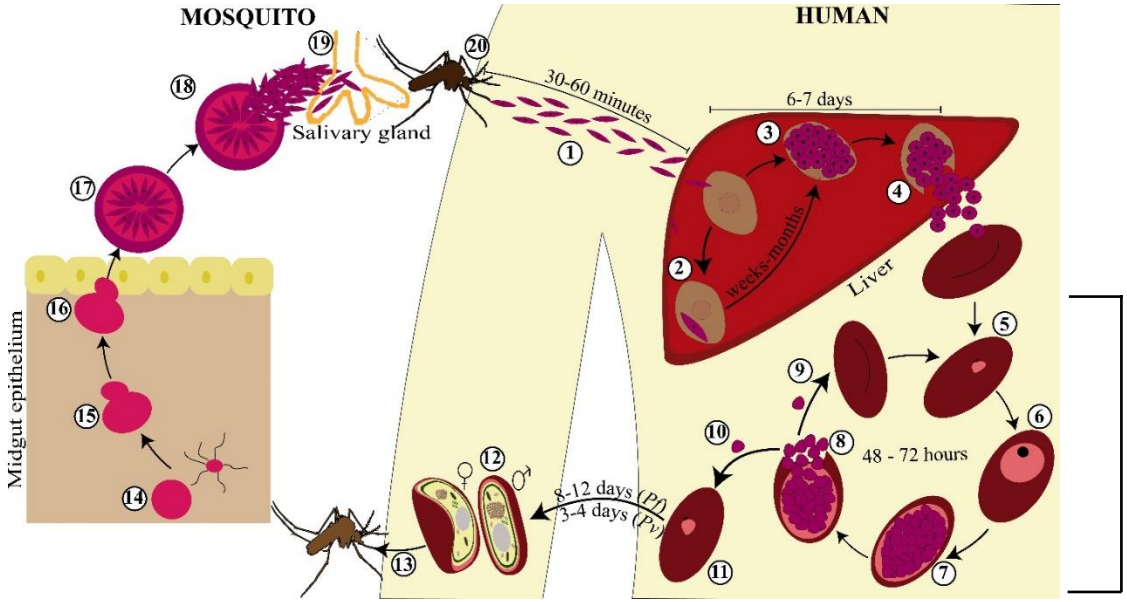


Fig1: Life cycle of the malaria parasite

A. Pre-erythrocytic phase: 1. Sporozoite injected by the mosquito, 2. Formation of hypnozoites (*P. vivax*), 3. Sporozoites dividing to form schizonts. **B. Exo-erythrocytic phase:** 4. Schizonts giving birth to thousands of merozoites released into the blood stream. **C. Erythrocytic phase:** 5. Ring stage, 6. Trophozoite stage, 7. Erythrocytic schizont, 8. Schizont giving birth to merozoites and released into the blood stream, 9. Merozoite targeting other erythrocytes. **D. Gametocytogenesis:** 10. Sexually committed merozoite targeting erythrocytes, 11. Sexually committed ring stage, 12. Formation of mature gametocytes. **E. Gametogenesis:** 13. Gametocytes taken up by mosquito, 14. Microgamete and Macrogamete, 15. Zygote, 16. Ookinete. **F. Sporogony cycle:** 17. Ookinete penetrating midgut wall of mosquito to form oocyst, 18. Oocyst ruptured to release sporozoites, 19. Sporozoites making way to mosquito salivary gland, 20. Infected mosquito biting human.

HOST FACTORS ON MALARIA SUSCEPTIBILITY

Gαs and β_2AR regulate the parasite entry into RBCs

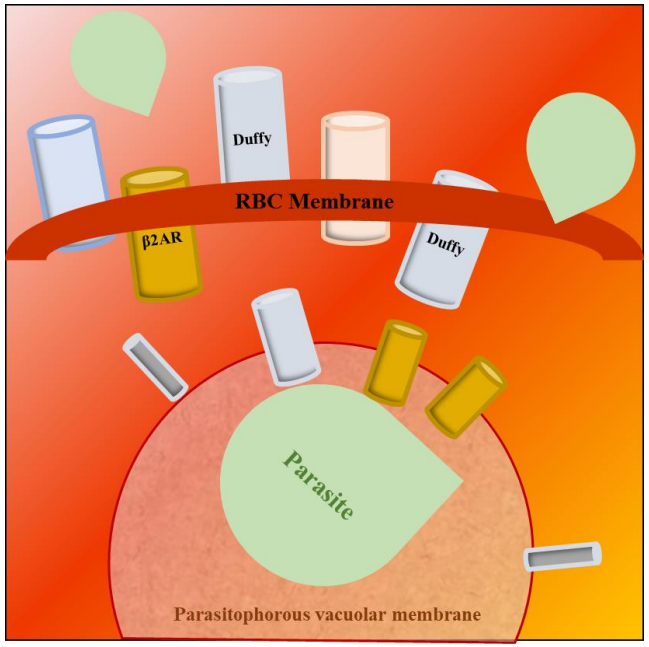
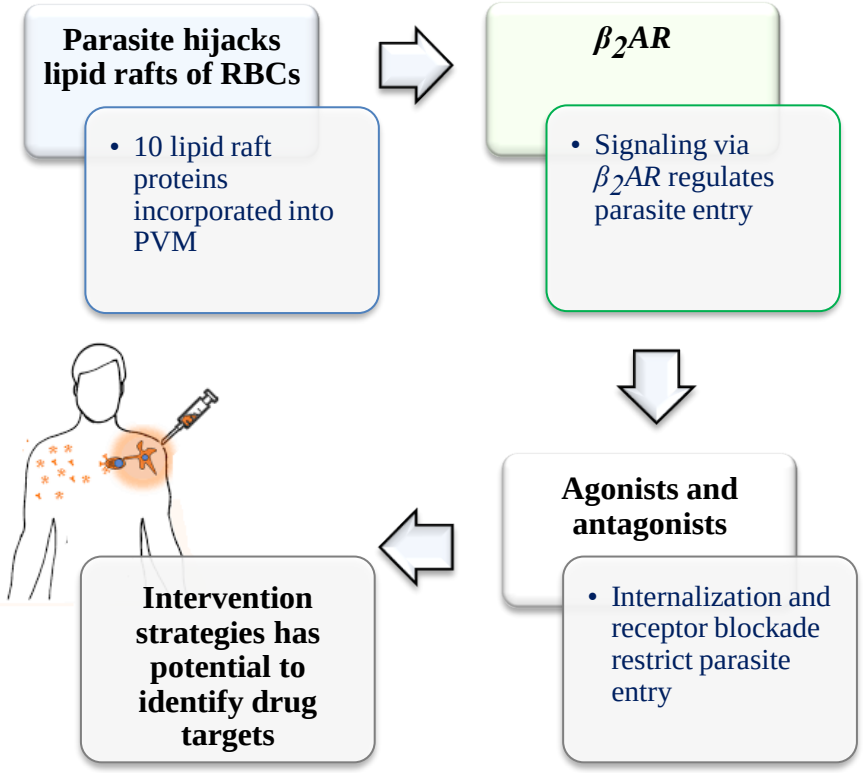
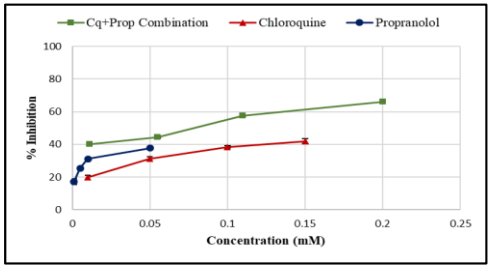
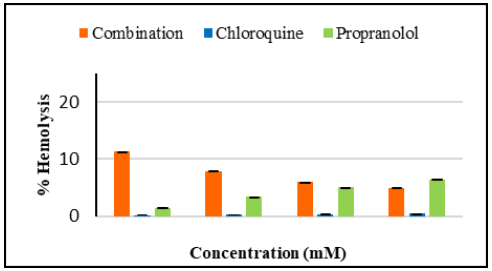


Figure 1: Erythrocyte membrane proteins characterization and selective uptake during malarial infection

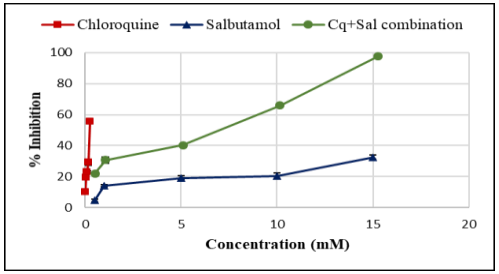


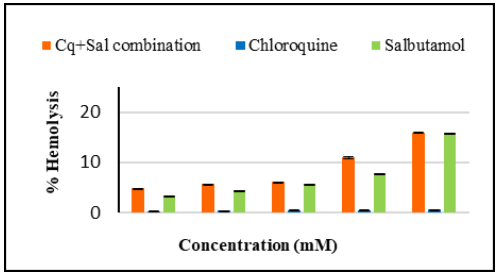
In vitro drug testing



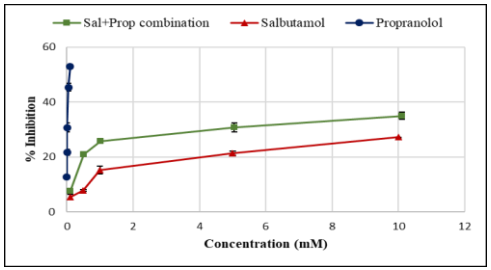


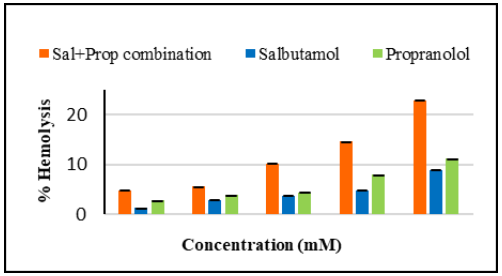
Assessment of inhibitory activity and hemolytic activity of Chloroquine and Propranolol





Assessment of inhibitory activity and hemolytic activity of Chloroquine and Salbutamol





Assessment of inhibitory activity and hemolytic activity of Salbutamol and Propranolol

Assessment of inhibitory activities and cytotoxicity of drugs on *P. falciparum in vitro*

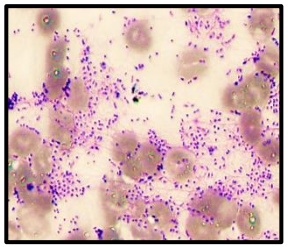


Fig 1: 29%I, 0.3%H

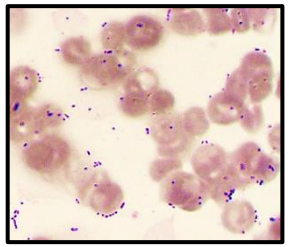


Fig 2: 97%I; 0.38H

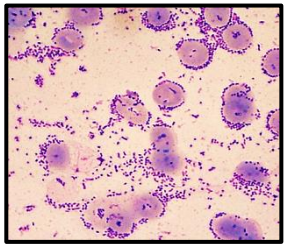


Fig 3: 30%I, 0.3%H

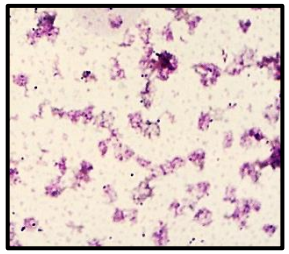


Fig 4: 90%I; 89%H

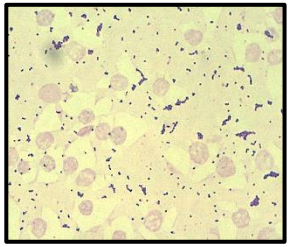


Fig 5: 41%I; 4%H

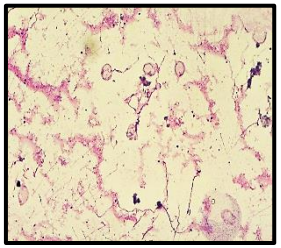


Fig 6: 88%I; 89%H

Microscopic observation of effects of drugs on *P. falciparum in vitro* (I: Inhibition, H: Hemolysis)

Agonist-induced desensitization of ADRβ2

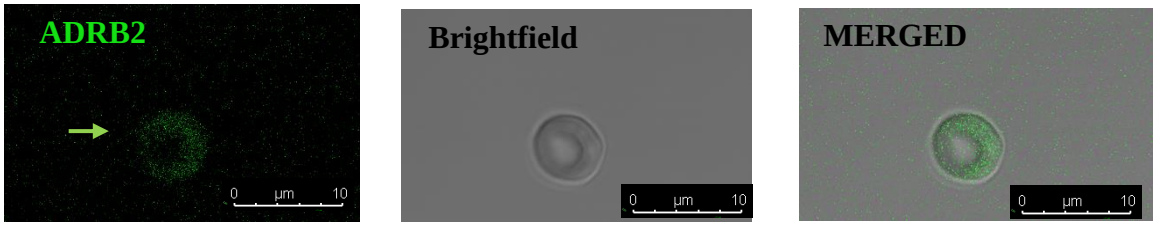


Figure 1: Visualization of control erythrocytes

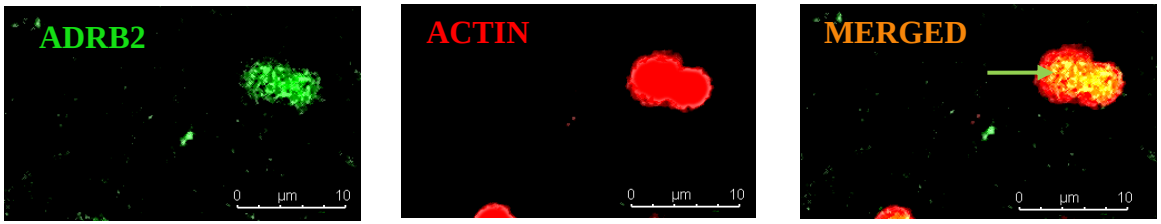


Figure 2: Salbutamol stimulated internalization of human ADRB2 receptors in erythrocytes

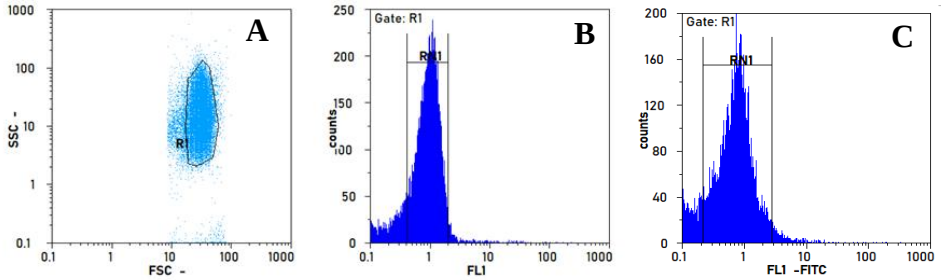


Figure 3: Flow cytometry analysis of anti-ADRB2 immunostaining of erythrocytes corresponding to the control cells (B) and the agonist treated cells (C)
A: R1: gate selecting the erythrocyte population among the events; B, C: Histogram representing the intensity of labeling

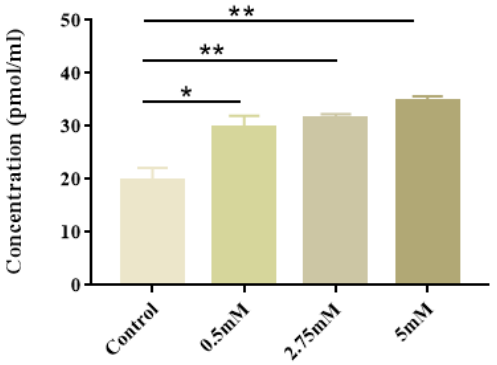
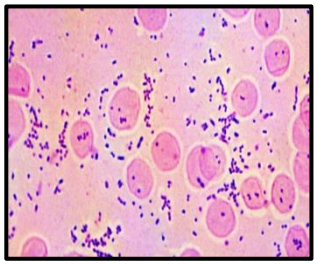
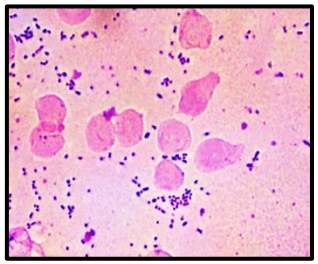


Figure 4: cAMP production in erythrocytes treated with agonist

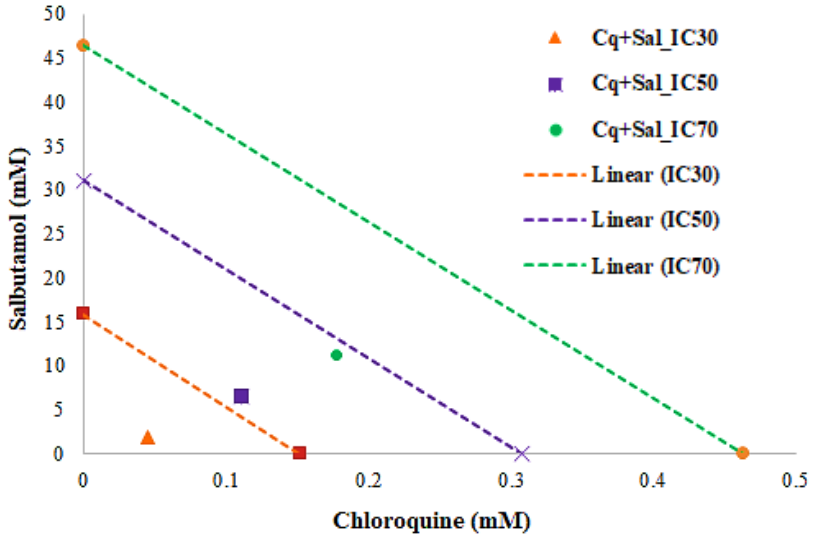
Conclusions



Parasite invaded red cells



Treated cells with agents that block invasion



- The measured inhibitory activity of the drugs *in vitro*, indicated that the antagonist propranolol showed more anti-plasmodial effect amongst the drugs tested
- Agonist treatment stimulated receptor internalization indicating loss of receptors for parasite invasion
- Rigorous *in vitro* experiments for anti-plasmodial dosimetry warrants further *in vivo* studies
- Repurposing of drugs appear to minimize therapeutic resistance to conventional treatment and increases effectiveness of the outcome as a strategy to counter progressive drug resistance. Malaria eradication call for improvements in treatment strategies as well as development of combination and alternate therapies

Acknowledgments

Dr. TMA Pai PhD Scholarship, MAHE, Manipal

MAHE Intramural Funding MAHE/DREG/PHD/IMF/2019

ICMR-SRF, Government of India

Technology Information Forecasting and Assessment Council (TIFAC)-Core in Pharmacogenomics, MAHE, Manipal

Fund for Improvement of S&T Infrastructure (FIST), Department of Science and Technology, Government of India

All participants of the study (IEC: 248/2019), Faculty members and Ms. Varijakshi G., MAHE, Manipal, India

Thank you!