Chemical Synthesis, Efficacy and Safety of Antimalarial Hybrid Drug Comprising of Sarcosine and Aniline Pharmacophores as Scaffolds

Authors:

1,5 Jean Baptiste Niyibizi, 2,3 Peter G. Kirira, 3 Francis Kimani, 4 Fiona Oyatsi, 1,4 Joseph K. Ng’ang’a

Affiliations:

1. Pan African University Institute of Sciences, Technology and Innovation, Nairobi, Kenya.
2. Mount Kenya University, Nairobi, Kenya.
3. Kenya Medical Research Institute, Nairobi, Kenya.
4. Jomo Kenyatta University of Agriculture and Technology, Nairobi, Kenya.
5. University of Global Health Equity, Kigali, Rwanda.

Corresponding author: niyibizi3@gmail.com, jniyibizi@ughe.org

ABSTRACT

Background: Reducing the impact of malaria is a key to achieving the sustainable development goals which are geared towards eradicating the disease. The main objective of this study was to synthesize, determine the efficacy and safety of antiplasmodial hybrid drug comprising of sarcosine and aniline derivative for management of plasmodial infections.

Results: The hybrid drug was synthesized by adding thionyl chloride to sarcosine in order to form acyl chloride which was then added to aniline to form sarcosine-aniline hybrid molecule. The IC50 of sarcosine-aniline hybrid was 44.80 ± 4.70 ng/ml compared to that of aniline derivative which was 22.86 ± 1.26 ng/ml. The IC50 of control drugs were 2.63 ± 0.38 ng/ml, 5.69 ± 0.39 ng/ml for artesunate and chloroquine respectively. There was a significant difference between IC50 of sarcosine-aniline hybrid and aniline derivative (P<0.05). There was also a significant difference between sarcosine-aniline hybrid and standard drugs used to treat malaria including artesunate and chloroquine (P<0.05). The ED50 of sarcosine-aniline hybrid drug was 6.49mg/kg compared to that of aniline derivative which was 3.61mg/kg. The ED50 of control drugs were 3.56 mg/kg, 2.94mg/kg and 1.78 mg/kg for artesunate-aniline hybrid, artesunate and chloroquine respectively. There was a significant difference (P<0.05) between ED50 of sarcosine-aniline hybrid and both controls such as aniline derivative, artesunate, artesunate-aniline hybrid and chloroquine. Cytotoxicity results revealed that sarcosine-aniline hybrid was safe to vero cells with a CC50 of 50.18±3.53µg/ml. Sarcosine-aniline hybrid was significantly less toxic compared to artesunate, chloroquine and doxorubicin. Sarcosine-aniline hybrid was also safe to mice.
Conclusion: Therefore, covalent bitherapy should be used in drug development for drug resistance mitigation.

Key words: Chemical synthesis, IC$_{50}$, ED$_{50}$, CC$_{50}$, Antimalarial resistance, Sarcosine-aniline hybrid drug.

1. Background

Chemotherapy has been the backbone of malaria control strategy. The plasmodia species that are considered responsible for malaria in humans are: *P. vivax*, *P. falciparum*, *P. ovale*, *P. malariae* and *P. knowlesi* (CDC, 2015). However, *P. falciparum*, the parasite that causes over 90% of all global malaria cases is more frequently becoming resistant to classical antimalarials, necessitating an urgent need for research and synthesis of new antimalarial agents, preferably with novel mode of action (Rosenthal, 2003). In the past two decades, only a few compounds belonging to a new class of antimalarial drugs, including aminoalcohols such as mefloquine, halofantrine and lumefantrine, sesquiterpene trioxanes such as artemisinin derivatives and naphthoquinones such as atovaquone were developed for clinical usage (Saifi *et al.*, 2013). Currently, artemisinin based combination therapy (ACT) is considered as the gold standard against *P. falciparum*, in which the regimen uses a double combination therapy geared towards delay of resistance, or preventing it altogether. Many approaches to antimalarial drug discovery deployed include optimization of therapy with available drugs such as: combination therapy, developing analogs of the existing drugs, evaluation of potent agents from natural products especially plants, use of compounds originally developed against other diseases, evaluation of drug-resistance reversers (chemosensitizers) as well as new chemotherapeutic targets (Rosenthal, 2003).

Currently, chemotherapy is the most important strategy to treat and prevent malaria. However, reports from Southeast Asia, indicate parasite resistance to artemisinin based which are considered as gold standard in treatment of malaria according to WHO (Wells *et al.*, 2015). This suggests the need to develop new antimalarial drugs that are effective against malaria parasite based on new mode of action, molecular targets and chemical structure. *In silico* studies have shown that sarcosine (N-methyl glycine) is able to bind to unique plasmodia proteins, hence inhibiting parasite growth. In addition, it was shown that 3-Chloro-4-(4-chlorophenoxy) aniline (aniline derivative) can bind to *P. f* ENR (Enoyl Acyl Carrier Protein Reductase), an enzyme
which catalyze the last step of the elongation cycle in the biosynthesis of fatty acids (Cronan, 2009) thereby inhibiting the parasite growth. Nevertheless, no experimental study has been conducted to confirm this aspect of the two to have antiplasmodial activity singly or in a hybrid molecule.

Globally, malaria transmission occurs in five WHO regions. It is estimated that 3.2 billion people in 95 countries and territories are at risk of being infected with plasmodium species and developing disease (figure 1), and 1.2 billion are at high risk, where more than 1 in 1000 people have chance of getting malaria in a year. Results from the World Malaria Report of 2015 show that the malaria cases were ranging between 149 to 303 millions or an average of 214 million cases of malaria globally. The African region accounted for most global cases of malaria (88%), followed by the South-East Asia region (10%) and the Eastern Mediterranean region (2%). In addition, the number of people that died of malaria was in the range of 236, 000 to 635, 000, representing an average of 438 000 malaria deaths. The high burden was heaviest in the WHO African Region (figure 1), where an estimated 90% of all malaria deaths usually occur (figure 1), and in children aged under 5 years, who accounted for more than two thirds of all deaths (WHO, 2015). This is because children of this age group are highly susceptible to infection, illness than adults (Snow et al., 2005). From the year 2000, malaria incidence rates fell by 37% globally, and by 42% in Africa whereas the malaria mortality rates went down by 66% in the African region and by 60% globally (Figure 1). (WHO, 2015).

(WHO, 2015)

Source: World malaria report 2015
Figure 1: Global distribution of Malaria throughout the world

Figure 1 shows that most of the malaria cases occur in Africa followed by Southeast Asia and Latin America. From the same figure, it is also shown that malaria has been eradicated in USA and Europe. Malaria remains a challenging disease in its diagnosis and control. Even though, there are some effective drugs for treatment of malaria, this could in the near future be lost to drug resistance in plasmodium species. Furthermore no effective vaccine has been successful so far (Doolan et al., 2003). The rapid emergence of *P. falciparum* strains resistance to the available antimalarial drugs and cross-resistance of parasites against antimalarial agents has resulted in the real need for new drugs to control malaria (Wells et al., 2015). Despite improvements in the malaria diagnosis and control, parasite resistance to antimalarial drugs continues to be a serious threat, hence the need for new antimalarial drugs. Reports from Southeast Asia, indicate possibility of parasite resistance to artemisinin based drugs, the current drug of choice in malaria chemotherapy, thus suggesting a novel development to malaria therapies. Many other antimalarial drugs of choice have developed resistance over time, for instance CQ resistance was documented on every continent with malaria in 1972-1990. In the late 1990s, mefloquine resistance emerged in malaria from Southeast Asia (Wells et al., 2015). In search for new drugs based on how the antimalarial drug discovery pipeline has changed over the past 10 years, it is urgent to look for new biological molecules that are effective against malaria parasite based on new mode of action, molecular targets and chemical structure (Figure 2). Sarcosine and aniline were suggested to have antiplasmodial activity based on bioinformatics studies. Sarcosine acts by binding to unique parasite’s proteins that play key roles in parasite survival whereas aniline can inhibit the Enoyl-acyl carrier protein (ACP) reductases (ENRs), which catalyzes the last step of the elongation cycle in the biosynthesis of fatty acids (Cronan, 2016). Covalent linking of sarcosine and aniline can carry a dual activity with different mode of action for inhibiting plasmodial growth. Based on the urgent need in drug development, it was necessary to validate this claim in this study. The expected outcome was that sarcosine-aniline hybrid could contain the dual activity of inhibiting the pyrimidine and fatty acids biosynthesis which might circumvent the parasite resistance to ACTs.
2. Methods

2.1 Study design
This project was a prospective lab based experimental study carried out at Kenya Medical Research Institute (KEMRI), Centre for Biotechnology Research and Development (CBRD), Malaria unit.

2.2 Materials

2.2.1 Sources of chemicals
Sarcosine, aniline, thionyl chloride, Dichloromethane, Magnesium sulfate and ammonium chloride, and Thin Layer Chromatography (TLC) plates provided by Sigma Aldrich, Kenya.

2.2.2 Experimental animals and parasites
Female Swiss albino mice were got and kept in the animal house of the Center for Biotechnology and Research Development at Kenya Medical Research Institute (KEMRI) where the animal experiments were carried out. The animals were six weeks old, weighing 20 to 22g, and they were kept in mice cages and were allowed to access mice pencils (Ungafeed Company Ltd) and water prior experimental testing. Plasmodium berghei ANKA strains, sensitive to quinolone and artemisinin based drugs were obtained from the Unit of Malaria, Centre for Biotechnology and Research and Development, Kenya Medical Research Institute. Parasites were maintained and cryopreserved in freezer (-80°C). The parasite was subsequently maintained in the laboratory by serial blood passage from mouse to mouse on weekly basis.

Figure 2: Structure of aniline and sarcosine
2.2.3 Vero Cells

Vero cells were obtained from Center for Traditional Medicine Drugs and Research (CTMDR), KEMRI. Vero cells are kidney cells extracted from an African green monkey (*Cercopithecus aethiops*). They were stored in nitrogen tank (-191°C) in Biological department, CTMDR, KEMRI.

2.2 Procedure for coupling aniline to sarcosine

Thionyl chloride (1.5 mL, 20 mmol) was added to sarcosine (0.09 g, 1.0 mmol) and the resulting suspension was refluxed for 6 hours to give a clear yellow solution. Excess thionyl chloride was removed *in vacuo* and the acid chloride was dissolved in dry dichloromethane (CH$_2$Cl$_2$) (10 mL) and cooled to 0°C. A solution of 3-Chloro-4-(4-chlorophenoxy) aniline (4.0 mmol) and triethylamine (0.20 mL, 2.0 mmol) in dry CH$_2$Cl$_2$ (2.5 mL) was added via cannula. The resulting mixture was stirred at room temperature for 16 hours during which time a white precipitate was formed. The suspension was washed with half-saturated aqueous ammonium chloride solution (2 × 6 mL) and water (2 × 3 mL), then dried over anhydrous magnesium sulphate (MgSO$_4$) and concentrated *in vacuo*. The formation of hybrid molecule was monitored by Thin Layer Chromatography (TLC).

2.3.2 Thin Layer Chromatography procedure

The TLC was run by spotting the sarcosine-aniline hybrid on TLC plate in a solvent system of ethyl acetate (2ml). The plate was dried in order to observe and take a photo of the spot, in a small container that has a lid mix of about 4g of silica gel and 1g of iodine crystals. The TLC plate was placed in the mixture and shaked gently in order to get in contact with the TLC plate for 5 minutes. The plate was taken to fluorescent machine under UV to observe dark spots of aniline and sarcosine-aniline hybrid. After TLC experiment, the location of each spot on the plate was represented by calculating its Retention factor (Rf). The Retention factor (Rf) was calculated by dividing the distance travelled by the compound by the distance from the baseline to the solvent front (Spangenberg *et al.*, 2011).

\[
Rf = \frac{\text{distance spot moved}}{\text{distance solvent moved}}
\]  
(Spangenberg *et al.*, 2011)
2.4 *In vivo* efficacy of sarcosine-aniline hybrid

2.4.1 Procedure for *in vivo* efficacy
This consisted of *in vivo* evaluation of hybrid drug against *P. berghei* ANKA, a rodent parasite which is commonly used in antimalarial studies (Fidock *et al.*, 2004). Swiss albino donor mouse was inoculated with *P. berghei* ANKA and parasitaemia was assessed after 5 days under microscope using giemsa staining technique. The donor mouse was sedated using carbon dioxide in order to collect blood via cardiac puncture in heparinized tube using a syringe and a needle. Carcasses were pooled in a bio-hazard container and stored at room temperature while waiting to be incinerated. Afterwards, the experimental mice were infected with inoculums of 1x10⁷ parasitized erythrocytes using intraperitoneal method. The mice were grouped into five groups consisting of 5 mice per group. The sarcosine-aniline hybrid was dissolved in 10% of tween-80 and daily oral drug administration was done for 4 days. Blood for making thin blood smear was collected from the tip of the mouse tail. The efficacy of the drug was measured by comparison of blood parasitaemia after the four days of therapy (that is on day 5 post-infection). The ones still alive at the end of the experiments were killed by sacrificing them using carbon dioxide gas followed by incineration.

2.4.2 Determination of the dose
The determination of the dose to be administered was calculated individually according to each mouse weight. In this regard, these formulas were applied:

\[ \text{HED (mg/kg)} = \text{Animal Dose (mg/kg)} \times \left( \frac{\text{Animal Km}}{\text{Human Km}} \right), \]

Where HED stands for Human Equivalent Dose and Km which is a conversion factor.

With Km (a constant) = Weight/Body surface area

For an adult human of 60kg, with body surface area of 1.6, Km= 60/1.6=37

For mice of 0.02kg, with body surface area of 0.007, Km= 0.02/0.007=3

(Shannon *et al.*, 2007).

\[ \text{Dosage (mg/kg)} = \frac{\text{Drug concentration (mg/ml)} \times \text{Volume (ml)}}{\text{Body weight (Kg)}}\]

From the above second formula:

\[ \text{Volume (ml) of drug to be given to each mouse} = \frac{\text{Body weight (Kg)} \times \text{Dosage (mg/kg)}}{\text{Drug concentration (mg/ml)}} \]

(Research Animal Resources Center, 2016)
2.4.3 Administration of the drug
Administration of the dose for testing drugs and reference drugs (artesunate and chloroquine) to assess the treatment of the experimental groups had been done using oral method, with varying dosages of 10, 5, 2.5, 1.25 and 0.625mg kg\(^{-1}\). The control groups had been given the normal saline alongside testing using the same procedure. The parasitaemia was checked for every 24 hours from time of infection for 4 days. Thin blood smears were prepared from each mouse tail venous blood. The smears were prepared by giemsa staining technique and the parasitaemia of individual mouse had been checked using light microscope (Kalra et al., 2006).

2.4.4 Procedure for giemsa staining
Prepared thin blood smears were fixed with methanol for 1-2 minutes after placing them on the staining rack at horizontal flat position. Smears were then flooded with Giemsa working solution and allowed to be stain for 10 to 20 minutes. After this, slides were rinsed with tap water and then dried on air dryer. Microscopy examination was done using light microscope for observation of parasitized red blood cells. The following formula was used to calculate the percentage parasitemia:

\[
\text{The Percentage of parasitaemia} = \frac{\text{Number of parasitized RBCs/field}}{\text{Total number of RBCs/field}} \times 100
\]

(Kalra et al., 2006)

After 4 days, the percentage (%) chemosuppression of each drug was determined using the formula:

\[
\text{Percentage chemosuppression} = 100 - \left(\frac{A - B}{A}\right) \times 100
\]

(Peters et al., 1975)

Where A is the mean parasitaemia in the negative control group and B is the parasitaemia in the test group. The dose that cured 50% of infected animals had been determined as Effective dose (ED\(_{50}\)) using a non-linear regression logistic dose-response model.

2.5 Cytotoxicity assays

2.5.1 Preparation of drugs solutions
To conduct in vitro toxicity, stock solutions of the test compound and reference drugs were prepared with sterile water. The drugs which were insoluble in water had their solubility been
enhanced by first dissolving 10mg in 100µl of 100% dimethyl-sulfoxide. The test samples were prepared as a 1mg ml⁻¹ stock solution. Further dilutions were prepared on the day of biological assays. All the drug solutions were stored at 4°C for later use.

2.5.2 Culture of the vero cells
Cells were maintained in Minimum Essential Eagles Medium (MEM) containing 10% fetal bovine serum (FBS), penstrep, and glutamine. Vero cells were cultured using T-75 culture flasks. The flasks were kept at 37°C in 5% CO₂ and the cells passaged every 2 to 3 days in order to keep the cells alive. Trypsinization was carried out to detached cells which overlap each other and counted using the hemocytometer counter (Neubauer).

2.5.3 Procedure for cytotoxicity assay
Using a 96 micro well plate, a cell density of 20,000 vero cells were seeded and incubated for 24 hours at 37 °C under 5% CO₂ to allow cells to attach to the surface/base of the plate.

Sarcosine-aniline hybrid drug as the test drug and controls were added in triplicate to the cultured cells using a concentration range 100µg/ml and subsequent dilutions were carried out over 7 folds, from 100, 50, 25, 12.5, 6.25, 3.125, 1.5625 to 0.78125 µg/ml. The plates were incubated for 48 hours at 37°C under 5% CO₂ to allow the reaction to occur. After 48 hours, 3-(4, 5-dimethylthiazol-2-yl)-2,5-diphenyltetra-zolium bromide (MTT) was added for calorimetric measurement of the ability of a drug to kill the vero cell lines. DMSO was added to each well and mixed to ensure cell lysis and dissolving of the formazan crystals.

Optical density was read using Multi Skan Ex reader machine 48X, from Thermo Fisher Scientific company in a UV–visible spectrophotometry at 562 and 690 nm. The amount of formazan measured was directly proportional to the number of viable cells. The results were recorded as optical density (OD) per well at each drug concentration and analyzed using Microsoft Excel software 2010, from which the percentage of cytotoxicity (PC) was calculated using the following equation:

\[
\text{Percentage cytotoxicity} = \left\{\frac{(A - B)}{A}\right\} \times 100
\]
(Mosmann, 1983).
Where A is the mean OD of untreated cells and B is the mean OD at each drug concentration. The drug concentration that leads to 50% inhibition of cell growth (CC$_{50}$) was determined by non-linear regression logistic dose-response model.

### 2.6 *In vivo toxicity studies*

In this step, acute toxicity was done. Three dosages, such as 2000 mg kg$^{-1}$ and 300 mg kg$^{-1}$ and 50mg/kg were administered orally to mice in three groups and the fourth group which served as control received water as a placebo. Each group consisted of 3 mice and the monitoring of weight and number of dead mice was monitored and recorded after each 4 days over an interval of 14 days. Clinical signs were also recorded during the study period. At the end of experiment, the mice were killed by sacrificing them using concentrated carbon dioxide gas followed by incineration.

The end points of the mouse experiment in the *in vivo* assays were set to consider with the development of the clinical signs such as impaired ambulation which prevents animals from reaching food or water, excessive weight loss and extreme emaciation, lack of physical or mental alertness, difficult labored breathing and prolonged inability to remain upright. To avoid severe and enduring distress mice that showed those clinical signs were killed through euthanasia using carbon dioxide gas into a closed chamber and then incinerated.

### 3. RESULTS

#### 3.1 Sarcosine-aniline hybridization

The sarcosine-aniline hybrid drug was synthesized by coupling sarcosine to aniline as shown in the following reactions:

\[
\begin{align*}
\text{H}_2\text{N} & \quad \text{O} \\
\text{O} & \quad \text{S} \\
\text{Cl} & \quad \text{Cl} \\
\text{C} & \quad \text{C} \\
\text{O} & \quad \text{H} \\
\text{O} & \quad \text{S} \\
\text{SO}_2 & + \quad \text{HCl}
\end{align*}
\]

**Figure 3:** Reaction between sarcosine and thionyl chloride

Figure 3 shows the reaction between sarcosine and thionyl chloride in dicloromethane solvent in order to form acyl chloride. This reaction is irreversible because SO$_2$ and HCl gasses that are lost in reaction mixture.
Figure 4: Coupling acyl chloride to aniline

Figure 4 illustrates the reaction between acyl chloride and aniline to form sarcosine-aniline hybrid at room temperature. In this reaction, there is a nucleophilic substitution of Cl by NH$_2$. The formation of sarcosine-aniline hybrid was spotted on thin layer chromatography and observed as band under UV light (Plate 1).

Plate 1: Thin Layer Chromatography results

Plate 1, shows the results of sarcosine-aniline hybrid formation on Thin Layer Chromatography plate. EtOAc stands for ethyl acetate and solvent system used to run this TLC consisted only of EtOAc (100% concentrated). Aniline was used as a control in the middle of the plate (second
column). From left to right, for the first and third column, the first spots closer to the base line of the columns is 3-Chloro-4-(4-chlorophenoxy) aniline, then the spots closer to the top are Sarcosine-aniline hybrid. The sarcosine-aniline hybrid is less polar than 3-Chloro-4-(4-chlorophenoxy) aniline, so it would elute (released) faster than 3-Chloro-4-(4-chlorophenoxy) aniline. The Retention factor (Rf) value for the sarcosine aniline hybrid (S-A) was 4.4/5.8=0.76 and the Rf of aniline was 4/5.8=0.69.

a. **In vitro activity of sarcosine-aniline hybrid**

**In vitro activity of 3-Chloro-4(4-chlorophenoxy) methyglycylaniide**

Table 1: IC$_{50}$ of sarcosine-aniline hybrid versus control drugs on *P. falciparum* 3D7 strains

<table>
<thead>
<tr>
<th>DRUGS</th>
<th>IC$_{50}$</th>
<th>P value</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>1</td>
</tr>
<tr>
<td>1 Sarcosine-aniline hybrid drug</td>
<td>44.80 ± 4.70 ng/ml</td>
<td>p≤0.001*</td>
</tr>
<tr>
<td>2 Aniline</td>
<td>22.86 ± 1.26 ng/ml</td>
<td>p≤0.001*</td>
</tr>
<tr>
<td>3 Artesunate</td>
<td>2.63 ± 0.38 ng/ml</td>
<td>p≤0.001*</td>
</tr>
<tr>
<td>4 Chloroquine</td>
<td>5.69 ± 0.39 ng/ml</td>
<td>p≤0.001*</td>
</tr>
<tr>
<td>5 Sarcosine</td>
<td>No activity</td>
<td></td>
</tr>
</tbody>
</table>

*p* significant: *P* $< 0.05$

Table 1 shows the activity of the drug which inhibits the growth of *P. falciparum* 3D7 strains, CQ sensitive. The IC$_{50}$ of sarcosine-aniline hybrid was 44.80 ng/ml. Sarcosine did not show *in vitro* activity. Table 1 also describes the comparisons of IC$_{50}$ of sarcosine-aniline hybrid drug against artesunate, aniline and chloroquine. There was a significant difference between the IC$_{50}$ of sarcosine-aniline hybrid drug against aniline, artesunate and chloroquine (*P* $< 0.05$).

3.3 **In vivo efficacy studies**

Table 2: ED$_{50}$ of sarcosine-aniline hybrid versus control drugs on *P. berghei* ANKA

<table>
<thead>
<tr>
<th>DRUGS</th>
<th>ED$_{50}$</th>
<th>P value</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>1</td>
</tr>
<tr>
<td>1 Sarcosine-aniline hybrid drug</td>
<td>6.49mg/kg</td>
<td>p≤0.001*</td>
</tr>
</tbody>
</table>
Table 2 shows the effective dose of the drug which inhibits the growth of *P. berghei* ANKA sensitive strain to quinoline and artemisinin based drugs *parasites* by 50% after four days of oral treatment. The ED$_{50}$ of sarcosine-aniline hybrid was 6.49mg/kg. The other drugs and compounds such as artesunate, artesunate-aniline hybrid, chloroquine and sarcosine were used as controls. Sarcosine did not show *in vivo* activity.

Table 2 also describes the comparisons of ED$_{50}$ of sarcosine-aniline hybrid drug against artesunate, artesunate-aniline hybrid, aniline and chloroquine. There was a significant difference between the ED$_{50}$ of sarcosine-aniline hybrid drug against artesunate, artesunate-aniline hybrid, and chloroquine (P <0.05).

![Figure 5: Percentage of parasitemia chemosuppression of sarcosine-aniline hybrid](image)

Figure 5 shows the percentage of chemosuppression of parasitaemia for sarcosine-aniline hybrid, aniline derivative, artesunate, artesunate-aniline hybrid and chloroquine after 4days of oral
treatment. The percentage of chemosupression for mice treated with 10mg/kg of sarcosine aniline hybrid was 52% whereas those treated with aniline, it was 68%. No parasites observed under microscope for the mice treated with 10mg/kg of artesunate and that of chloroquine.

Table 3: Parasitemia growth of sarcosine-aniline hybrid after stopping treatment

<table>
<thead>
<tr>
<th>Drugs</th>
<th>Day 7</th>
<th>Day 9</th>
<th>Day 11</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sarcosine-aniline hybrid</td>
<td>9.74</td>
<td>12.98</td>
<td>15.61</td>
</tr>
<tr>
<td>Aniline</td>
<td>6.97</td>
<td>9.03</td>
<td>11.84</td>
</tr>
<tr>
<td>Artesunate</td>
<td>0.00</td>
<td>0.2</td>
<td>0.67</td>
</tr>
<tr>
<td>CQ</td>
<td>0.00</td>
<td>0.03</td>
<td>0.42</td>
</tr>
</tbody>
</table>

Table 3 shows the actual average of parasitemia at day 7, 9 and 11 after stopping the treatment with 10mg/kg. The parasitemia of sarcosine-aniline hybrid was 9.74%, 12.98% and 15.61% on day 7, 9 and 11 respectively. The recrudescence of CQ and artesunate reappeared on day 9 with 0.03% and 0.2% respectively.

Table 4: Survival rate after 11 days of in vivo efficacy testing

<table>
<thead>
<tr>
<th>Dosage</th>
<th>Sarcosine-aniline hybrid</th>
<th>Sarcosine</th>
<th>3-Chloro-4-(4-chlorophenoxy) aniline</th>
<th>Artesunate</th>
<th>Artesunate-aniline hybrid</th>
<th>Chloroquine</th>
</tr>
</thead>
<tbody>
<tr>
<td>10mg/kg</td>
<td>3</td>
<td>1</td>
<td>4</td>
<td>5</td>
<td>3</td>
<td>5</td>
</tr>
<tr>
<td>5mg/kg</td>
<td>2</td>
<td>2</td>
<td>2</td>
<td>3</td>
<td>2</td>
<td>4</td>
</tr>
<tr>
<td>2.5mg/kg</td>
<td>1</td>
<td>1</td>
<td>3</td>
<td>4</td>
<td>3</td>
<td>4</td>
</tr>
<tr>
<td>1.25mg/kg</td>
<td>1</td>
<td>1</td>
<td>3</td>
<td>3</td>
<td>3</td>
<td>3</td>
</tr>
<tr>
<td>0.625mg/kg</td>
<td>1</td>
<td>0</td>
<td>1</td>
<td>2</td>
<td>1</td>
<td>3</td>
</tr>
</tbody>
</table>

Table 4 shows the number of mice which survived up to 11 days of experiment, which is 7 days after stopping oral drug administration. For sarcosine-aniline hybrid, 3 mice among 5 survived for the groups which received 10 mg/kg, and 2 mice survived in group which received 5 mg/kg whereas only 1 mouse survived for the groups which received 2.5 mg/kg, 1.25 mg/kg and
0.625mg/kg. Up to day 11, all mice which received 10mg/kg of artesunate and chloroquine survived along \textit{in vivo} efficacy testing.

3.4 \textit{In vivo} toxicity

Acute toxicity

Table 5: Number of dead mice following different dosage administrations

<table>
<thead>
<tr>
<th>Group</th>
<th>Dosage of hybrid drug administered orally</th>
<th>Number of mice per group</th>
<th>Number of dead mice after 28 days</th>
</tr>
</thead>
<tbody>
<tr>
<td>Group 1</td>
<td>2000mg/kg</td>
<td>3</td>
<td>0</td>
</tr>
<tr>
<td>Group 2</td>
<td>300mg/kg</td>
<td>3</td>
<td>0</td>
</tr>
<tr>
<td>Group 3</td>
<td>50mg/kg</td>
<td>3</td>
<td>0</td>
</tr>
<tr>
<td>Control group</td>
<td>0mg/kg</td>
<td>3</td>
<td>0</td>
</tr>
</tbody>
</table>

Table 5 illustrates the number of dead mice during acute toxicity testing for 14 days. The three groups of mice received different dosage (2000 mg/kg, 300 mg/kg, 50 mg/kg) of sarcosine-aniline hybrid drug and the control group did not receive any dosage rather water. For all groups, the initial weights were ranging from 20 to 22g and they were all females. No mouse died in all groups for 14 days.

Table 6: Weights of mice according to hybrid drug dosage during acute toxicity experiment

<table>
<thead>
<tr>
<th>Groups</th>
<th>Dosage</th>
<th>Mice</th>
<th>Day 1</th>
<th>Day 5</th>
<th>Day 9</th>
<th>Day 14</th>
<th>Mean</th>
<th>SD</th>
<th>Mean average</th>
</tr>
</thead>
<tbody>
<tr>
<td>Group 1</td>
<td>2000mg/kg</td>
<td>M1</td>
<td>20</td>
<td>19.4</td>
<td>20.1</td>
<td>21.5</td>
<td>20.25</td>
<td>0.88</td>
<td>21.34</td>
</tr>
<tr>
<td></td>
<td></td>
<td>M2</td>
<td>21</td>
<td>20.5</td>
<td>22.0</td>
<td>24</td>
<td>21.87</td>
<td>1.54</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>M3</td>
<td>22</td>
<td>21</td>
<td>21.5</td>
<td>23.1</td>
<td>21.90</td>
<td>0.89</td>
<td></td>
</tr>
<tr>
<td>Group 2</td>
<td>300mg/kg</td>
<td>M1</td>
<td>20</td>
<td>19.7</td>
<td>20.6</td>
<td>21.7</td>
<td>20.50</td>
<td>0.88</td>
<td>21.58</td>
</tr>
<tr>
<td></td>
<td></td>
<td>M2</td>
<td>21</td>
<td>20.3</td>
<td>21.9</td>
<td>23.1</td>
<td>21.57</td>
<td>1.20</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>M3</td>
<td>22</td>
<td>21.6</td>
<td>22.7</td>
<td>24.3</td>
<td>22.69</td>
<td>1.19</td>
<td></td>
</tr>
<tr>
<td>Group 3</td>
<td>50mg/kg</td>
<td>M1</td>
<td>20</td>
<td>20.6</td>
<td>21.2</td>
<td>23.4</td>
<td>21.3</td>
<td>1.48</td>
<td>22.24</td>
</tr>
<tr>
<td></td>
<td></td>
<td>M2</td>
<td>21</td>
<td>21.4</td>
<td>22.5</td>
<td>24.2</td>
<td>22.27</td>
<td>1.43</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>M3</td>
<td>22</td>
<td>21.5</td>
<td>24.0</td>
<td>25.1</td>
<td>23.15</td>
<td>1.69</td>
<td></td>
</tr>
<tr>
<td>Placebo</td>
<td></td>
<td>M1</td>
<td>20</td>
<td>20.7</td>
<td>21.0</td>
<td>23.5</td>
<td>21.17</td>
<td>1.28</td>
<td>22.33</td>
</tr>
</tbody>
</table>
Table 6 describes the weight of each individual mouse in each group and the weight variation in for 14 days of acute toxicity testing. Three groups of mice received different dosage (2000 mg/kg, 300 mg/kg, 50 mg/kg) of sarcosine-aniline hybrid drug and the control group did not receive any dosage rather water. The initial weights for all groups were ranging from 20 to 22g and they were all females. The weights were recorded after 4 days of intervals. The mean average of weight for the mice which received 2000 mg/kg was 21.34g after 14days. The mean average of weights for the mice which received 300 mg/kg, 50 mg/kg and control group were 21.58g, 22.24g and 22.33g respectively after 14 days of acute toxicity testing (table 6).

### Table 7: Multiple comparison of mice’s weights among groups after acute toxicity testing

<table>
<thead>
<tr>
<th>Groups of mice to be compared according to dosage administered</th>
<th>Mean difference</th>
<th>Significance</th>
</tr>
</thead>
<tbody>
<tr>
<td>Group1 (2000mg/kg) VS Group2 (300mg/kg)</td>
<td>-0.24667</td>
<td>0.990</td>
</tr>
<tr>
<td>Group1 (2000mg/kg) VS Group3 (50mg/kg)</td>
<td>-0.90000</td>
<td>0.714</td>
</tr>
<tr>
<td>Group1 (2000mg/kg) VS Control group (placebo)</td>
<td>-0.99000</td>
<td>0.654</td>
</tr>
<tr>
<td>Group2 (300mg/kg) VS Group3 (50mg/kg)</td>
<td>-0.65333</td>
<td>0.862</td>
</tr>
<tr>
<td>Group2 (300mg/kg) VS Control group (placebo)</td>
<td>-0.74333</td>
<td>0.812</td>
</tr>
<tr>
<td>Group3 (50mg/kg) VS Control group (placebo)</td>
<td>0.09000</td>
<td>1.000</td>
</tr>
</tbody>
</table>

*significant: P value < 0.05

Table 7 shows comparisons between groups of mice which received different dosages from the highest (2000mg/kg) to the lowest (50mg/kg) as well as control group which did not receive drug. There was no significant difference between mice which received 2000mg/kg and mice which received 300mg/kg (P value of 0.990), 50mg/kg (P value of 0.714) and control group (P value of 0.654), the drug and between the control group.

### 3.5 Cytotoxicity results

#### Table 8: The CC50 values of the different antimalarial drugs against vero cells

<table>
<thead>
<tr>
<th>SN</th>
<th>DRUGS</th>
<th>Mean CC50 in µg/ml</th>
<th>SD</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Sarcosine-aniline hybrid</td>
<td>50.18</td>
<td>3.53</td>
</tr>
<tr>
<td>2</td>
<td>Chloroquine</td>
<td>57.96</td>
<td>3.85</td>
</tr>
</tbody>
</table>
Table 8 shows the CC<sub>50</sub>, Standard deviation (SD) of the mean of sarcosine-aniline hybrid drug against vero cells after triplicate experiments. 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) assay was employed which is based on the ability of a mitochondrial dehydrogenase enzyme from viable cells to cleave the tetrazolium rings of pale yellow MTT and thereby form dark blue formazan crystals which are largely impermeable to cell membranes, resulting in their accumulation within healthy cells’cytoplasm. Chloroquine and artemisinin were used as standard drugs which are used to treat malaria whereas doxorubicin was used as a known cytotoxic drug. During experiment, the starting concentration (highest) for both drugs was 100µg/ml. Sarcosine-aniline hybrid showed a CC<sub>50</sub> of 50.18µg/ml while the CC<sub>50</sub> of doxorubicin was 1.96µg/ml.

If CC<sub>50</sub> of a drug is lesser than 2µg/ml, a drug is considered as cytotoxic, when CC<sub>50</sub> is 2-89µg/ml, a drug is considered as moderately cytotoxic and when it is above 90µg/ml, a drug is considered as not cytotoxic (safe) (Engineering, n.d. Retrieved from http://www.who.int/tdr/grants/workplans/en/cytotoxicity_invitro.pdf, on 10th March 2017)

Table 9: The CC<sub>50</sub> values of sarcosine-aniline hybrid versus other antimalarial drugs

<table>
<thead>
<tr>
<th>SN</th>
<th>Cell type to be compared</th>
<th>Mean Difference</th>
<th>P value</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Vero Sarcoise-aniline hybrid VS Vero Chloroquine</td>
<td>-7.78</td>
<td>0.006*</td>
</tr>
<tr>
<td>2</td>
<td>Vero Sarcoise-aniline hybrid VS Vero Artesunate</td>
<td>30.48</td>
<td>p≤0.001*</td>
</tr>
<tr>
<td>3</td>
<td>Vero sascosine-aniline hybrid VS Vero Doxorubicin</td>
<td>48.21</td>
<td>p≤0.001*</td>
</tr>
</tbody>
</table>

*significant: P < 0.05

Table 9 describes the comparisons of CC<sub>50</sub> of sarcosine-aniline hybrid drug against artemisinin, chloroquine and doxorubicin. There was a significant difference between the CC<sub>50</sub> of sarcosine-aniline hybrid drug against chloroquine, artemisinin and doxorubicin. The highest mean difference was observed between the CC<sub>50</sub> of sarcosine-aniline hybrid and doxorubicin (48.21) whereas the lowest mean difference was observed between sarcosine-aniline hybrid and chloroquine (-7.78).

**Therapeutic index of sarcosine-aniline hybrid**

Therapeutic index (TI) of drug is equal to its lethal dose concentration divided by its effective dose (LD50/ED50). The LD<sub>50</sub> of sarcosine-aniline hybrid was estimated to be greater than 5000mg/kg
and its ED50 was 6.49 mg/kg. Therefore the Therapeutic Index of sarcosine-aniline hybrid drug was greater than 770.41.

4. DISCUSSION

4.1 Sarcosine-aniline hybridization

In this study, the two molecules were used to synthesize a hybrid: 3-Chloro-4-(4-chlorophenoxy) aniline and sarcosine. The molecular purity of 3-Chloro-4-(4-chlorophenoxy) aniline and sarcosine was 97% and 98% respectively as confirmed by the manufacturer labels upon their delivery in Kenya by Sigma Aldrich. The sarcosine was completely soluble in water but the solubility of 3-Chloro-4-(4-chlorophenoxy) aniline and the hybrid in water was very low. They were soluble in dimethyl sulfoxide (DMSO), and slightly soluble in Tween-80. It is clear that the low solubility of sarcosine-aniline hybrid affected the drug absorption, distribution and its metabolism. Confirmed by thin layer chromatography, the hybrid (was successfully synthesized using 3-Chloro-4-(4-chlorophenoxy) aniline and sarcosine pharmacophores.

4.2 In vivo efficacy of sarcosine-aniline hybrid

Results from this study showed that sarcosine-aniline hybrid drug has in vivo antimalarial activity. However, sarcosine, when used singly was not having inhibition activity against *P. berghei* ANKA sensitive strain (ED50 not detectable). This was due to different reasons, such as sarcosine being transformed into glycine by glycine-N-methyl transferase enzyme. The ED50 of sarcosine-aniline hybrid drug was 6.49 mg/kg whereas that of 3-Chloro-4-(4-chlorophenoxy) aniline alone was 3.61mg/kg. There was a significant difference between ED50 of sarcosine-aniline hybrid and both controls such as 3-Chloro-4-(4-chlorophenoxy) aniline, artesunate, artesunate-aniline hybrid drug and chloroquine. On the hand, there was no significant difference between ED50 of artesunate-aniline hybrid drug and 3-Chloro-4-(4-chlorophenoxy) aniline as well as between 3-Chloro-4-(4-chlorophenoxy) aniline and artesunate. In the study done on in vivo antiplasmodial potentials of the combinations of four Nigerian antimalarial plants extracts, the ED50 of CQ using oral administration pathway was 2.2 mg/kg (Adebajo et al., 2014), and this is in agreement with current study where the ED50 of chloroquine was 1.78 mg/kg.
The *in vivo* activity of sarcosine-aniline hybrid drug was less than that of other control drugs used in this study. This may be due to other conversions of the hybrid drug which may occur along oral administration pathway such as in the bowels. As a result this can prevent hybrid molecule from reaching the target sites. The type of drug administration method for effectiveness of a drug can also be of a great concern. Therefore, if sarcosine-aniline hybrid drug can be administered intravenously or subcutaneously, it might also improve its efficacy as well as its bioavailability. It is known that the artesunate has a shorter half-life and its combination with other drugs increases its bioavailability. The half-life of both 3-Chloro-4-(4-chlorophenoxy) aniline and sarcosine is not yet known in order to determine their stability while combined or hybridized with other compound or drugs. Artesunate-aniline hybrid drug was active than both 3-Chloro-4-(4-chlorophenoxy) aniline and artesunate when used singly. This might be because artesunate is a semi-synthetic derivative of artemisinin whose water solubility facilitates absorption and provides an advantage over other artemisinins. In addition, artesunate is rapidly hydrolyzed to dihydroartemisinin, which is the most active schizonticidal metabolite (Li & Weina, 2010). In contrast, 3-Chloro-4-(4-chlorophenoxy) aniline being a low water soluble compound might affect the artesunate solubility in resultant hybrid drug.

A study done on potent *in vivo* anti-malarial activity and representative snapshot pharmacokinetic evaluation of artemisinin-quinoline hybrid, the three synthesized artemisinin-quinoline hybrids were differing from each other by one methyl group in the linker and position in the chain, their $ED_{50}$ were 1.1, 1.4 and <0.8 mg/kg respectively using intraperitoneal route and 12, 16 and 13 mg/kg respectively using oral route (Lombard *et al.*, 2013). The $ED_{50}$ of artesunate was 1.8 mg/kg using oral route with 80mg/kg as highest dosage and it was <1mg/kg using intraperitoneal route (Lombard *et al.*, 2013), these results differ slightly with my results because of the dosages and methods used, in current study, no intraperitoneal method used rather oral method. In another study done on oral artesunate dose-response relationship in acute Falciparum malaria, the effective doses were determined after treating patients from whom the infections were detected with a dose varying from 0 to 250 mg of artesunate together with a curative dose of mefloquine, the resultant $ED_{50}$ was 1.6 mg/kg (Angus *et al.*, 2002), this result differ with current study as the artesunate here was combined with mefloquine and the study used the patients while in my study I used *P.berghei* in mice.
The percentage of chemosuppression for sarcosine-aniline hybrid was around 55% whereas that of aniline was 68%, the percentage of chemosuppression was 100% for mice treated with 10mg/kg of artesunate and chloroquine. However, there was a recrudescence after 5 days post treatment for both mice treated with artesunate and chloroquine whereas for other drugs, there was a continuous increase of the parasitemia after stopping the treatment (Table 2). The recrudescence in mice treated with artesunate and chloroquine, might be due to some remnant parasites which survived after stopping the treatment. In the study done on antimalarial activity of methanolic leaf extract of *piper betle* L, there was 100% of chemosuppression for the mice treated with chloroquine with 20 mg/kg using oral route (Abdulelah, *et al.*, 2010), which agrees with current study where the percentage of chemosuppression was 100% at day four following oral treatment at 10 mg/kg.

The number of mice which survived up to 11days of experiment during in vivo efficacy testing was recorded. Actually after 7days when the drug administration was stopped, the number of mice which were still alive was different according to the type of drug and its dosage. This is because at this dosage there was a 100% of parasites’ growth inhibition. According to current study, there was where the low drug dosage showed a high number of survived mice compared to the higher dosage concentration of the same drug.

### 4.3 In vitro toxicity of sarcosine-aniline hybrid

*In-vitro* toxicity tests are the alternative method approaches to animal acute toxicity evaluation. Vero cell lines are usually employed in prospective studies to determine the cytotoxic effect of different natural and artificial products (Menezes *et al.*, 2013). Cytotoxicity results from this study, showed that the hybrid drug was safe to vero cells. When compared to other standard drugs, sarcosine-aniline hybrid was significantly less toxic compared to artesunate. There was also a significant difference between cytotoxicity of hybrid drug and that of CQ against mammalian cell lines. In the study done on effects of chloroquine to inhibit dengue virus type 2 replication in vero cells but not in C6/36 cells, the concentrations equal or greater than 500 µg/ml showed major cytotoxicity but the the concentrations equal or lesser than 50µg/ml did not reveal cytotoxicity effects on vero cells (Kleber *et al.*, 2013). These results agree with study, where the CC_{50} of chloroquine was 57.96µg/ml.
Therefore, the side effects caused by CQ such as neurotoxicity, leukopenia, retinopathy and cardiovascular toxicity might be absent, reduced or increased for sarcosine-aniline hybrid drug, but this requires chronic toxicity to be done using mice so that blood parameters and histopathology of liver, kidney, heart and brain dysfunction might be elucidated. Doxorubicin was significantly more toxic than hybrid drug to vero cells. Doxorubicin served as a control in cytotoxicity studies as it was revealed that it is more toxic to cancerous cells and even to normal cells including vero cells which had been used in this study. Doxorubicin’s cytotoxicity is based on its capacity to bind to DNA-associated enzymes (topoisomerases), intercalate with DNA base pairs, and target multiple molecular targets in order to produce a wide range of cytotoxic effects. It also activates the Bcl-2/Bax apoptosis pathway when it interacts with cells’ membranes (Tacar, Srimornersak, & Dass, 2013).

4.4. Acute toxicity of sarcosine-aniline hybrid

Acute toxicity showed that the hybrid drug was safe to mice. There was no dead mice observed with 2000mg/kg, so the LD\textsubscript{50} is expected to be above 5000mg/kg, thus this drug is classified in category 5 according to OECD/OCDE guideline for testing chemical compounds (OECD/OCDE, 2001). The weights measured during acute toxicity testing showed that the weight loss was observed in first week of experiment and increased in the second week of experiment for the group which received 2000mg/kg and 300mg/kg. There was a continuous gain in weights for the group which received a single dose of 50mg/kg and control group. There was no significant difference in weights within and between groups at the end of acute toxicity experiment. No clinical physical signs of discomfort such as impaired ambulation, excessive weight loss, lack of physical or mental alertness, difficult breathing, prolonged inability to remain upright and extreme emaciation observed during acute toxicity experiment. Therapeutic index of a drug is the ratio that compares the blood level concentrations at which a given drug is toxic (lethal dose) and the concentration at which the drug is effective (effective dose), which is a vital criteria for drug selection (Xie et al., 2006).

Therapeutic index of sarcosine-aniline hybrid drug is greater than 770.41, which confirms its safety as the concentration required to cause toxicity (>5000mg/kg) is far greater than that of required to kill the parasites (6.49 mg/kg) (Muller & Milton, 2012). The closer the TI is to 1, the more dangerous is and the larger the therapeutic index (TI) the safer the drug is (Muller & Milton, 2012). In fact, if the TI is small, the drug might be dosed thoroughly and the patient receiving the drug...
should be monitored carefully for any clinical signs of drug toxicity (Muller & Milton, 2012). The study done by Xie et al., (2006), on new potential antimalarial agents: Therapeutic-Index evaluation of Pyrroloquinazolinediamine and its prodrugs in a rat model of severe malaria, the therapeutic index of artesunate was 4 (Xie et al., 2006). When compared the results from Xie et al., (2006), the therapeutic index of sarcosine-aniline hybrid is 192.6 folds greater than that of artesunate which implies that the sarcosine-aniline hybrid is safer than artesunate.

5. Conclusions
Sarcosine-aniline hybrid has been synthesized using sarcosine and 3-Chloro-4-(4-chlorophenoxy) aniline pharmacophores and the product formation was monitored and confirmed by Thin Layer Chromatography. Sarcosine-aniline hybrid drug is a promising antiplasmodial prodrug as it showed activity for in vitro and in vivo studies with an IC50 of 44.80 ± 4.70 ng/ml and an ED50 of 6.49 mg/kg, which are within acceptable ranges of drugs used to treat severe malaria (WHO, 2015). This study points out that sarcosine-aniline hybrid drug is safe to vero cells with CC50 of 50.18±3.53µg/ml compared to doxorubicin which is most toxic with CC50 of 1.96±0.59µg/ml. The acute toxicity results showed no dead mice up to the dosage of 2000mg/kg using oral administration for 14 days, and there was no significant loss of weight in mice within and between groups with different dosages of sarcosine-aniline hybrid as well as control group. There should be use of covalent bitherapy in drug development. The use of covalent biotherapy in drug resistance mitigation is recommended.

6. List of abbreviations
1. **ACT**: Artemisinin Combination Therapy
2. **Aniline**: 3-Chloro-4-(4-chlorophenoxy) aniline
3. **ATCase**: Aspartate Carbamyl-transferase
4. **CC50**: Cytotoxicity Concentration at 50%
5. **CDC**: Center for Disease Control and Prevention
6. **CQ**: Chloroquine
7. **DMSO**: Dimethyl sulfoxide
8. **ED50**: Effective Dose at 50%
9. **ENR**: Enoyl Acyl Carrier Protein Reductase
10. **IC50**: 50% inhibitory concentration
11. **IP:** Intraperitoneal
12. **IC**$_{50}$: Inhibition Concentration at 50%
13. **JVKUAT:** Jomo Kenyatta University of Agriculture and Technology
14. **KEMRI:** Kenya Medical Research Institute
15. **MKU:** Mount Kenya University
16. **MTT:** 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide
17. **MEM:** Minimum Essential Eagle’s Medium
18. **OD:** Optical Density
19. **OECD:** Organization for Economic Co-operation and Development
20. **PAUSTI:** Pan African University, Institute of Basic Sciences Technology and Innovation
21. **P. f AT Pase:** P. falciparum sarco-endoplasmic reticulum calcium ATPase
22. **P. f ENR:** (Enoyl Acyl Carrier Protein Reductase)
23. **PMI:** President’s Malaria Initiative
24. **RBCs:** Red Blood Cells
25. **SERU:** Scientific and Ethics Review Unit
26. **WHO:** World Health Organization.

7. **Declarations**

Ethics approval and consent to participate

Handling of animals was done in accordance to the KEMRI Guide for Care and Use of Laboratory animals. Ethical clearance was sought from Scientific Ethic Review Unit (SERU), in KEMRI (Protocol number: KEMRI/SERU/CBRD/PROP155/3324). Mice were handled with a lot of care as stipulated in guidelines set by KEMRI Animal Care and Use Committee (Ref Number: KEMRI/CBRD/MP/AH/01).

Consent for publication

Not applicable

Availability of data and material

The datasets used and/or analyzed during the current study are available from the corresponding author.
Competing interests

The authors declare no conflicts of interests as far as this publication is concerned.

Funding

This project was funded by Pan African University, Institute of basic sciences and Innovation through African Union (AU). AU funded in the design of the study and collection, analysis, and interpretation of data and in writing the manuscript.

Authors' Contributions

JBN conducted in vitro, in vivo experiments and drafted the manuscript. FO, PGK and JBN worked together to synthesize the hybrid molecule. KF provided the facilities for in vitro testing. JKN and PGK revised the work and approved it. All authors have read and approved the manuscript.

Acknowledgements

Authors are also grateful to Kenya Medical Research and Mount Kenya University for providing some crucial facilities during laboratory work. This work was presented in Joint 5th SASA international conference & 2nd Rwanda biotechnology conference and in 2nd world congress & expo on biotechnology and bioengineering. The funding agency of this project did not lead to any conflict of interests regarding the publication of this manuscript.

Authors’ Information

Jean Baptiste Niyibizi, Head of Science Laboratory and Lecturer in Basic Medical Sciences division, MBBS/MGHD, University of Global Health Equity, Rwanda. He holds Bachelor of Science in Biomedical Laboratory Sciences and Master of Science in Molecular Biology and Biotechnology.

Prof Joseph Kangethe Ng’ang’a: He is a professor of Biochemistry at Jomo Kenyatta University of Agriculture and Technology, He holds PhD in Biochemistry

Dr Peter G. Kirira is a senior Lecturer in Physical sciences department at Mount Kenya University. He holds PhD in Chemistry.
Dr Francis Kimani: He is the Head of Malaria research department at Kenya Medical research institute (KEMRI). He holds PhD in Biochemistry.

Fiona Oyatsi: She is a research assistant at Mount Kenya University, and she holds Master of Science in Medicinal Chemistry.

8. References


