Supporting information

Aryl-alkyl-lysines: Small Molecular membrane active antiplasmodial agents

Chandradhish Ghosh, a Shweta Chaubey, b Utpal Tatu a and Jayanta Haldar a*

a Chemical biology and Medicinal Chemistry Laboratory, New Chemistry Unit, Jawaharlal Nehru Centre for Advanced Scientific Research (JNCASR), Jakkur, Bengaluru 560064, Karnataka, India.
b Department of Biochemistry, Indian Institute of Science, Bengaluru, India

Table of Contents

Page S2: Materials and instrumentation
Page S3-S14: Synthesis and Details of Characterization
Page S15-S18: Biological assays
Page 19: Supplementary figures
Page 20: References
Materials and instrumentation

All the solvents were of reagent grade and were distilled and dried prior to use wherever required. Chloroform and methanol were supplied by Merck-India. Dimethylformamide, Dichloromethane, Diethyl ether and other solvents were supplied either by SDFCL (India) or Spectrochem (India). L-Lysine, Di-tert-butyl carbonate, Diisopropylethylamine, HBTU, Hexylamine, Benzaldehyde, Trifluoroacetic acid were purchased from Spectrochem (India). 1-Naphthaldehyde, 2-Naphthaldehyde were purchased from Sigma-Aldrich. All the chemicals were used as supplied. 4-Quinolinecarboxaldehyde and Biphenyl-4-carboxaldehyde were bought from Alfa Aesar. Analytical thin layer chromatography (TLC) was performed on E. Merck TLC plates pre-coated with silica gel 60 F254 (250 μm thickness). Visualization was accomplished using UV light and Iodine. Column chromatography was performed on silica gel (60-120 Å pore size). HPLC analysis was performed on a Shimadzu-LC 8A Liquid Chromatograph instrument (C18 column, 10 mm diameter, 250 mm length) with UV detector monitoring at 254 nm. Nuclear magnetic resonance spectra were recorded on Bruker (AV-400) 400 MHz spectrometer in deuterated solvents. Infrared (IR) spectra of the solid compounds were recorded on Bruker IFS66 V/s spectrometer using KBr pellets. IR spectra of the compounds soluble in low-boiling solvents were recorded with the same instrument using NaCl crystal. High-resolution Mass Spectrometry was recorded on Agilent 6538 Q-TOF LC-MS system and Shimadzu LC-MS 2020 spectrometer. Optical density was measured by TecanInfinitePro series M200 Microplate Reader. Fluorescence images were taken using a Zeiss 510 Meta confocal laser-scanning microscope. The images were prepared using LSM 5 Image examiner. The animal experiments were performed at Indian Institute of Science and JNCASR following the institute guidelines.
Synthesis and details of characterization.

General procedure for synthesis of \(N\)-alkylaminomethylarene hydrochlorides (1a-7a)

These compounds were prepared using the protocol published earlier.\(^1\) Briefly, in a mixture of dry chloroform and methanol (10 ml), aromatic aldehydes (1.27 mmol) and alkyl amines (1.27 mmol) were stirred at room-temperature (under Nitrogen atmosphere) for 6 h. At the end of 6h, the solution was cooled to 0°C before adding sodium borohydride (2.3 mmol) was added. This was stirred at room temperature for 12h. Subsequently, the solvents were evaporated under reduced pressure (not to dryness) and diluted with diethyl ether. 2N NaOH (20 ml) was added to it and stirred for 15 minutes. The organic layer was then separated from the NaOH layer and was subsequently washed with water twice, brine and dried over MgSO\(_4\). The organic layer was then evaporated under reduced pressure to dryness before dissolving the residue in minimum volume of methanol. 4N HCl (3 ml) was added to it to obtain precipitates of the salt. The solvent was completely removed and the precipitate was dissolved in minimum volume of ethyl acetate (a few drops of methanol was added to dissolve completely). To this hexane was added to obtain pure crystals of the target compound (Yield: 70-90%). These crystals were filtered, dried and subsequently characterized using \(^1\)H NMR, and Mass spectrometry.

The details of characterization of compounds 1a-4a have been previously reported and are not furnished here.\(^1\)

\(N\)-(quinolin-4-ylmethyl)hexan-1-aminium chloride (5a): Yield-70%. \(^1\)H NMR (400 MHz, CDCl\(_3\)) \(\delta/\text{ppm}: 9.2\ (\text{Ar-CH}_2-NH_2-C_6\text{H}_{13}, 2\text{H}), 8.4-8\ (\text{Ar-H}, 6\text{H}), 5.1\ (s, \text{Ar-CH}_2-NH_2-, 2\text{H}), 3.3\ (t,
-NH₂-CH₂-C₅H₁₁, 2H), 1.8 (m, -NH₂-CH₂-CH₆H₄, 2H), 1.5-1.3 (m, -NH₂-C₂H₅-(CH₂)₃-CH₃, 6H), 0.82 (t, -NH₂-C₅H₁₂-CH₃, 3H). HR-MS (m/z): [M+H]+ obsd. = 243.1853 (calc. = 243.1861).

N-(naphthalen-2-ylmethyl)hexan-1-aminium chloride (6a): Yield-85%. ¹H NMR (CDCl₃) δ/ppm: 9.9 (s, Ar-CH₂-NH₂-C₆H₁₃, 2H), 8.1-7.51 Ar H, 7H), 4.5 (s, Ar-CH₂-NH₂-, 2H), 2.75 (t, -NH₂-CH₂-C₅H₁₁, 2H), 1.85 (q, -NH₂-CH₂-CH₂-C₄H₉, 2H), 1.2 (m, -NH₂-C₂H₅-(CH₂)₃-CH₃, 6H), 0.82 (t, -NH₂-C₅H₁₂-CH₃, 3H). HR-MS (m/z): [M+H]+ obsd. = 242.1889 (calc. = 242.1903).

N-([1,1'-biphenyl]-4-ylmethyl)hexan-1-aminium chloride (7a): Yield-76%. ¹H NMR (CDCl₃) δ/ppm: 9.9 (m, Ar-CH₂-NH₂-C₆H₁₃, 2H), 7.7 (d, ArH, 2H), 7.6 (d, ArH, 2H), 7.51 (m, ArH, 2H), 7.44-7.3 (ArH, 3H) 4.0 (s, Ar-CH₂-NH₂-, 2H), 2.8 (t, -NH₂-CH₂-C₅H₁₁, 2H), 1.87 (m, -NH₂-CH₂-CH₂-C₅H₁₂, 2H), 1.3-1.17 (-NH₂-C₂H₅-(CH₂)₃-CH₃, 6H), 0.82 (t, -NH₂-C₅H₁₂-CH₃, 3H). HR-MS (m/z): [M+H]+ obsd. = 268.2059 (calc. = 268.2065).

General procedure for amide coupling

Amide coupling was performed using a previously published scheme.¹ Preparation and characterization of Boc-Lys(Boc)-OH was also reported earlier.¹ In 5:2 DMF/CHCl₃, Boc-Lys(Boc)-OH (0.33 g, 0.95 mmol), N,N-Diisopropylethylamine (DIPEA, 420 µL, 2.37 mmol) was first added at 0°C. Subsequently, HBTU (0.36 g, 0.95 mmol) was added to the solution. This mixture was cooled to 0°C and subsequently the abovementioned secondary amines (0.79 mmol) were added to it. The mixture was initially stirred at 0°C for 30 minutes and then at RT for 24 h typically. At the end, organic layer was evaporated under reduced pressure before diluting to twice its original volume by addition of ethyl acetate. This ethylacetate layer was subsequently washed with 0.5 M KHSO₄, H₂O (thrice) and brine. After passage through anhydrous Na₂SO₄, the organic layer was evaporated under reduced pressure and the residue was purified using column
chromatography (5% MeOH/CHCl₃) to obtain the product in 65%-90% yield. The purified compound was subsequently characterized using ¹H NMR, IR and Mass spectrometry.

The details of characterization of compounds 1b-4b have been previously reported and are not furnished here.¹

**Boc-Lys(Boc)-N-(quinolin-4-ylmethyl)hexan-1-amide (5b):** Yield-72%. ¹H NMR (CDCl₃) δ/ppm: 8.15-7.9 (ArH, 2H), 7.8-7.5 (ArH, 2H), 7.1 (ArH, 1H), 5.2-4.9 (Ar-CH₁H₂-N(R)Lys(boc)₂ and α-NH-Boc of Lys(boc)₂, 2H), 4.75-4.40 (Ar-CH₁H₂-N(R)Lys(boc)₂,Lys (ε-NH-Boc)-α-NH-boc and α-CₖH₆ of Lys(boc)₂, 3H), 3.40-3.0 (δ-CH₂ of Lys(boc)₂ and Ar-CH₂-N(-C₅H₁₀-C₃H₁₁)Lys(boc)₂, 3H), 1.72-1.12 (-CO-[CH-CH₂-CH₂-CH₂-NH-CH₂-NH-COO-C(C₃H₃)₂]-NH-COO-C(C₃H₃)₃ of Lys(boc)₂ and –CH₂-(CH₂)₄-CH₃ of R group, 32H), 0.84 (m, Ar-CH₂-N(-C₅H₁₀-C₃H₃)Lys(boc)₂, 3H). FT-IR (cm⁻¹): 3331 (carbamate N-H str.), 3085 (sp² C-H str.), 2967-2867 (sp³ C-H str.), 1709 (C=O str. of tertiary amide), 1517-1450 (aromatic C=C str.). HR-MS (m/z): [M+H]⁺ obsd. = 570.3954 (calc. = 570.3907).

**Boc-Lys(Boc)-N-(naphthalen-2-ylmethyl)hexan-1-amide (6b):** Yield-80%. ¹H NMR (CDCl₃) δ/ppm: 7.86-7.74 (ArH, 3H), 7.62 (s, ArH, 1H) 7.5-7.42 (ArH, 2H), 7.32-7.3 (ArH, 1H), 5.4 (α-NH-Boc of Lys(boc)₂, 1H) 5.0-5.0 (Ar-CH₁H₂-N(R)Lys(boc)₂ and α-NH-Boc of Lys(boc)₂, 2H), 4.8-4.40 (Ar-CH₁H₂-N(R)Lys(boc)₂, Ar-CH₁H₂-N(R)Lys(boc)₂,Lys (ε-NH-Boc)-α-NH-boc and α-CₖH₆ of Lys(boc)₂, 4H), 3.50-2.9 (δ-CH₂ of Lys(boc)₂ and Ar-CH₂-N(-CH₂-C₃H₁₁)Lys(boc)₂, 4H), 1.76-1.15 (-CO-[CH-CH₂-CH₂-CH₂-CH₂-NH-COO-C(CH₂)₂]-NH-COO-C(CH₃)₂ of Lys(boc)₂ and –CH₂-(CH₂)₄-CH₃ of R group, 32H), 0.84 (m, Ar-CH₂-N(-C₅H₁₀-CH₂)Lys(boc)₂, 3H). FT-IR (cm⁻¹): 3331 (carbamate N-H str.), 3085 (sp² C-H str.), 2967-2867 (sp³ C-H str.), 1709 (C=O str. of carbamate), 1643 (C=O str. of tertiary amide), 1517-1450 (aromatic C=C str.). HR-MS (m/z): [M+H]⁺ obsd. = 570.3954 (calc. = 570.3907).
Boc-Lys(Boc)-N-([1,1'-biphenyl]-4-ylmethyl)hexan-1-amide (7b): Yield - 76%. \(^1\)H NMR (CDCl\(_3\)) \(\delta/\text{ppm}: 7.64-7.5\) (Ar\(H\), 4H), 7.48-7.38 (Ar\(H\), 2H), 7.38-7.3 (Ar\(H\), 1H), 7.3-7.2 (Ar\(H\), 2H) 5.38 (\(\alpha-NH\)-Boc of Lys(boc), 1H), 4.87-4.37 (Ar-CH\(^2\)H\(^2\)-N(R)Lys(boc), 2H, Ar-CH\(^1\)H\(^2\)-N(R)Lys(boc), 2H, Lys(\(\varepsilon-NH\)-Boc)-\(\alpha-NH\)-boc and \(\alpha-CH\) of Lys(boc), 4H), 3.50-2.9 (\(\delta-CH\) of Lys(boc) and Ar-CH\(_2\)-N(-CH\(_3\), 4H), 1.76-1.19 (\(-\text{CO-}[\text{CH-CH}]-\text{CH}-\text{CH}-\text{CH}-\text{CH}-\text{NH-COO-C(}CH\(_3\))\) of Lys(boc) and \(-\text{CH}-\text{CH}\)) of R group, 32H), 0.84 (m, Ar-CH\(_2\)-N(-C\(_5\)H\(_{10}\)-CH\(_3\))Lys(boc), 3H). FT-IR (cm\(^{-1}\)): 3331 (carbamate N-H str.), 3085 (sp\(^2\)C-H str.), 2967-2867 (sp\(^3\)C-H str.), 1709 (C=O str. of carbamate), 1643 (C=O str. of tertiary amide), 1517-1450 (aromatic C=C str.). HR-MS (m/z): [M+H]\(^+\) obsd. = 570.3954 (calc. = 570.3907).

**General procedure for deprotection of Boc groups (5-7)**

The deprotection of Boc groups were performed using a previously published protocol.\(^1\) Briefly, compounds (1b-7b) were dissolved in DCM and subsequently CF\(_3\)COOH (50% by volume) was added and stirred at RT. The reactions were monitored by TLC until complete removal of starting material was observed. All the volatile components were removed, and the product was purified by reverse phase HPLC using 0.1% Trifluoroacetic acid (TFA) in water/acetonitrile (0-100%) as mobile phase to more than 95% purity. C\(_{18}\) column (10mm diameter, 250 mm length) and UV detector (at 270 nm wavelength) were used. After drying the compounds in freeze drier, the compounds were characterized by \(^1\)H NMR, \(^{13}\)C NMR, IR and mass spectrometry. (All spectra are furnished in supplementary figures). Peptoids of naphthalene have been reported to show presence of rotamers in solution.\(^2\) These compounds too show existence of rotamers, as is evident from their
NMR spectra (furnished below in Supplementary Figures). For the same reason, the $^{13}$C spectra are not assigned.

**Lys-N-(quinolin-4-ylmethyl)hexan-1-amide trifluoroacetate (5):**

$^1$H NMR (400MHz, D$_2$O) $\delta$/ppm: 8.9 (ArH, 1H) 8.3-7.8 (Ar$H$, 4H) 7.5 (Ar$H$, 1H), 5.5-5.1 (Ar-$CH_2$-N(R)Lys, 2H), 4.7 (m, $\alpha$-CH of Lys, 1H), 3.8-2.7 (Ar-$CH_2$-N($CH_2$($CH_2$)$_2$CH$_3$)Lys and $\varepsilon$-$CH_2$ of Lys, 4H), 2.1-1.19 ($\beta$-$CH_2$ of Lys, $\gamma$-$CH_2$ of Lys, $\delta$-$CH_2$ of Lys and Ar-$CH_2$-$N($CH$_2$($CH_2$)$_2$C$_3$H$_7$)Lys and Ar-$CH_2$-$N($C$_2$H$_4$($CH_2$)$_2$CH$_3$)Lys, 14H), 0.85 (m, Ar-$CH_2$-$N($C$_5$H$_{10}$($CH_2$)$_2$Lys, 3H). FT-IR (cm$^{-1}$): 3414 (primary amine N-H str.), 3089 (sp$^2$ C-H str.), 2967-2867 (sp$^3$ C-H str.), 1678 (C=O str. of tertiary amide), 1517-1450 (aromatic C=C str.). HR-MS (m/z): [M+H]$^+$ obsd. = 371.2795 (calc. = 371.2811).
HPLC trace of compound 5.

$^1$H NMR spectra of compound 5 in D$_2$O. Solvent peak was assigned at 4.79 ppm.
$^{13}$C NMR spectra of compound 4 in CD$_3$OD.

HRMS spectra of compound 5
Lys-N- N-(naphthalen-2-ylmethyl)hexan-1-amide trifluoroacetate (6)

\[
\text{HPLC trace of compound 6.}
\]
$^1$H NMR spectra of compound 6 in D$_2$O. Solvent peak was assigned at 4.79 ppm.

$^{13}$C NMR spectra of compound 6 in CD$_3$OD.
HRMS spectra of compound 6.

**Lys-N-([1,1'-biphenyl]-4-ylmethyl)hexan-1-amide trifluoroacetate (7)**

![Chemical Structure](image)

$^1$H NMR (D$_2$O) δ/ppm: 7.37-7.12 (Ar$H$, 4H) 7.12-6.87 (Ar$H$, 5H), 4.5-4.1 (Ar-CH$^1$H$^2$-N(R)Lys, Ar-CH$^1$H$^2$-N(R)Lys and α-CH of Lys, 3H), 3.1-2.74 (Ar-CH$^2$-N(CH$_2$CH$_2$CH$_3$)Lys and ε-CH$_2$ of Lys, 4H), 1.94-1.19 (β-CH$_2$ of Lys, γ-CH$_2$ of Lys, δ-CH$_2$ of Lys and Ar-CH$_2$-N(CH$_2$CH$_2$C$_3$H$_7$)Lys, 8H), 0.8 (m, Ar-CH$_2$-N(C$_2$H$_4$C$_2$H$_2$CH$_3$Lys, 6H), 0.5 (m, Ar-CH$_2$-N(C$_5$H$_{10}$CH$_2$Lys, 3H). FT-IR (cm$^{-1}$): 3414 (primary amine N-H str.), 3089 (sp$^2$ C-H str.), 2967-2867 (sp$^3$ C-H str.), 1678 (C=O str. of tertiary amide), 1517-1450 (aromatic C=C str.). HR-MS (m/z): [M+H]$^+$ obsd. = 396.2995 (calc. = 396.3015).
HPLC trace of compound 7.

\[ \text{\textsuperscript{1}H NMR spectra of compound 6 in D}_2\text{O. Solvent peak was assigned at 4.79 ppm.} \]
$^{13}$C NMR spectra of compound 7 in CD$_3$OD.

HRMS spectra of compound 7.
Biological assays

Plasmodium falciparum culturing. Plasmodium falciparum 3D7 was cultured in human O+ erythrocytes in complete RPMI 1640 (Sigma Aldrich) medium supplemented with 0.5% albumax II (Invitrogen), 0.2% NaHCO3, 0.2% Glucose, 200μM Hypoxanthine and 5μg/L Gentamycin. At every 24 h, the spent medium was removed and replenished with fresh medium. The culture was scaled up by splitting it, once the parasitemia had increased up to 10%. A fresh O-positive human erythrocytes was added for the newer infection.

In vitro antimalarial activity

For in-vitro antimalarial screening, SYBR green I based fluorescence assay was used as reported by Smilkstein et al. with a minor modification. Parasites were synchronized to ring stages (haematocrit: 2%, parasitaemia: 1%, 100 μl) by sorbitol treatment. The compounds were serially diluted in complete RPMI media. Parasite cultures (50μL) were incubated with increasing concentrations of compounds in media (50μL) in a 96 well plate. The highest concentration tested was 10μM. CQ was used as positive controls and absence of compound was considered to be negative control. After 48 hr of incubation 100 μl of lysis buffer containing SYBR Green I was added to the 96 wells. The composition of the lysis buffer per mL is as follows: 0.2 μl of 10,000 X SYBR Green I (Sigma), Tris (20 mM; pH 7.5), EDTA (5 mM), saponin (0.008%; wt/vol), and Triton X-100 (0.08%; vol/vol). This solution (200μL) was then mixed gently with multi-channel pipette and incubated in dark at 37°C for 1 h. At the end of the incubation period, the solutions were transferred to black-round bottomed 96 well plates. Fluorescence was measured using a Tecan plate reader with excitation and emission wavelengths centred at 485 and 530 nm respectively. The fluorescence intensities were plotted against increasing compound
concentrations and IC\textsubscript{50} (the 50\% inhibitory concentration) was determined by analysis of sigmoidal curves. Giemsa-stained smears of compound-treated parasite cultures were also done in order to validate the results.

**Haemolytic activity.**

Hemolytic experiments were performed with slight modification of a previously reported protocol. Erythrocytes were isolated from freshly drawn, heparinized human blood and re-suspended to 5 vol\% in PBS (pH 7.4). In a 96-well microtiter plate, 150 \( \mu \)L of erythrocyte suspension was added to 50 \( \mu \)L of serially diluted compound. Two controls were made, one without compound and other with 50 \( \mu \)L of 1 vol\% solution of Triton X-100. The plate was incubated for 1 h at 37°C. The plate was then centrifuged at 3,500 rpm for 5 min, 100 \( \mu \)L of the supernatant from each well was transferred to a fresh microtiter plate, and \( A_{540} \) was measured. Percentage of hemolysis was determined as \( \frac{(A - A_0)}{(A_{total} - A_0)} \times 100 \), where \( A \) is the absorbance of the test well, \( A_0 \) the absorbance of the negative controls (without compound), and \( A_{total} \) the absorbance of 100\% hemolysis wells (with Triton X-100), all at 540 nm. The HC\textsubscript{50} values and errors are reported as averages and standard errors of mean of at least two independent experiments (each experiment was performed in triplicates) respectively. The error of the experiments is less than 10\%.

**In vitro stage dependence of action**

The stage dependence of action of the compounds were determined by addition of the compounds at specific stages of parasite development: ring, trophozoite and schizonts. To achieve a synchrony in stage development of *P. falciparum*, selective destruction of trophozoite and schizont stage erythrocytes by 5\% sorbitol was used.\textsuperscript{4} The parasites were tightly synchronized in the ring stages
using two rounds of sorbitol synchronization spaced 4h apart. This culture was divided into three groups. Each group was subdivided into two groups. In the subdivision, one served as treated while the other as positive control. The three groups were divided as rings, trophozoites and schizonts. Compounds were added at concentrations twice their IC$_{50}$ values to the ring stage culture after second synchronization. In case of trophozoites the culture was allowed to grown for 24 hr before addition of compounds. In case of schizonts the compounds were allowed to grow for 38 hr before addition of compounds. Rings, trophozoites and schizonts were allowed to grow for 48h, 24h, and 12h respectively before Giemsa-stained smears from the assays were analyzed microscopically. The ability of the compound to inhibit parasite development at different stages was gauged by comparing the numbers with untreated controls.

**Mechanism of action (Microscopy)**

For these experiments two dyes were used Rhodamine 123 and Lysotracker Green. Briefly, In a total volume of 200 $\mu$L, Parasite-infected erythrocytes (2% haematocrit and 3% parasitemia) were incubated at 37°C for 30 min with 1 $\mu$M rhodamine 123 (for parasite plasma membrane potential), or 10 nM LysoTracker Green (for DV potential) before incubation with the test compound for 4 hr. Untreated cultures served as negative controls. These were then transferred onto a cover slip, which was fixed using paraformaldehyde. The fixed coverslips were then visualized under a confocal microscope.

**In-vivo toxicology**

The *in-vivo* toxicology study was performed at JNCASR following the institute guidelines. For conducting in-vivo toxicology studies, the compounds (in PBS) were injected intravenously into female Balb/c mice (6-8 weeks old).
Systemic toxicity and LD$_{50}$ determination: Mice were treated with decreasing concentrations of compound 3 (in PBS) intravenously. The concentrations used were 175mg/kg, 55 mg/kg, 17.5 mg/kg and 5.5 mg/kg. Survival of mice were observed and the parameters were fit into Spearman-Karber’s equation to obtain the LD$_{50}$ values of the compounds.$^5$ Systemic toxicity was also examined after i.v injection of compounds. Animals were directly inspected for adverse effects for 4 h, and mortality was observed for 14 days.

Acute toxicity: For the evaluation of the acute toxicity, two groups of 10 mice each received intravenous injection of NCK-6 at 17.5 mg/kg in 0.2 ml of sterilized PBS. 10 mice were sacrificed at 48 h and the rest mice at 14 days to collect blood samples for analysis of biochemical parameters such as alanine transaminase (ALT), urea nitrogen, creatinine, sodium ion, potassium ion and chloride ion levels. Blood samples were analyzed at Gokula Metropolis clinical laboratory, Bengaluru, India.

**In-vivo activity**

Experimental studies with mice were conducted adhering to the institution’s guidelines for animal husbandry at the Indian Institute of Science. *P. berghei* ANKA Mouse Studies: 6-8 weeks old Swiss mice (female) were first injected with $10^7$ *P. berghei* ANKA parasitized RBCs via IP administration. Two hours after the infection, mice (n=4) were given dosages of Compound 3 (20 mg/kg) or PBS (Control) intraperitoneally (IP). These dosages were continued every 24 hours for four days. Parasitemia were determined on day 5 via Giemsa-stained smears of blood drawn from the tail of the mice. Survival of the mice were monitored till they were dead. Mice experiments were performed at Indian Institute of Science following the guidelines of the institute.
Fig. S1: Images of parasites treated with compound 3 at the end of the *Plasmodium* lifecycle. The treatment was initiated at A) Trophozoite stage B) Schizont stage. The figure clearly shows that the compound was not effective at these stages.
References


