Chapter 3

Synthesis, antimalarial activity and cytotoxicity of 10-aminoethylether derivatives of artemisinin - Article 1

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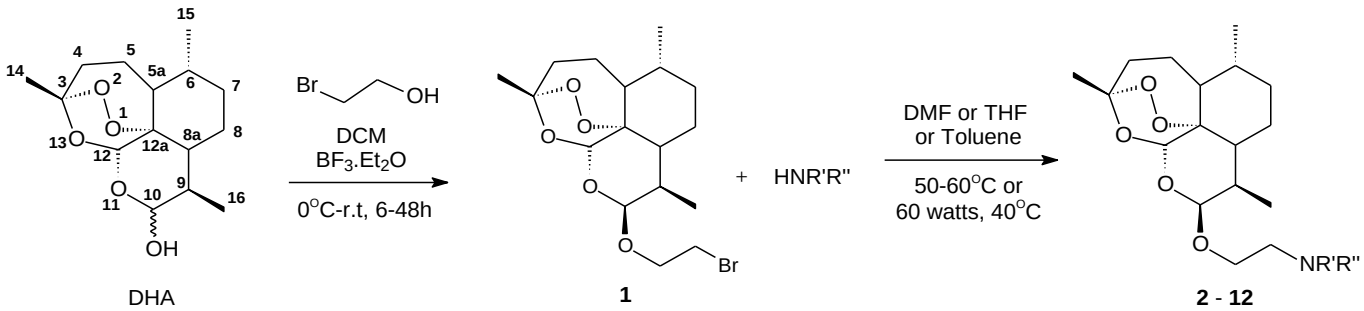
Graphical abstract



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Abstract

In this study, a series of 11 10-aminoethylether derivatives of artemisinin were synthesised and their antimalarial activity against both the chloroquine sensitive (D10) and resistant (Dd2) strains of *Plasmodium falciparum* was determined. The compounds were prepared by introducing aliphatic, alicyclic and aromatic amine groups with linkers of various chain lengths through an ethyl ether bridge at C-10 of artemisinin using conventional and microwave assisted syntheses, and their structures were confirmed by NMR and HRMS. All derivatives proved to be active against both strains of the parasite. The highest overall activity was displayed by the short chain aromatic derivative **8** (IC_{50} = 1.44 nM), containing only one nitrogen atom, while long chain polyamine derivatives were found to have the lowest activity against both strains. An interesting correlation between the IC_{50}, pK_a values and resistance index (RI) was found.

Keywords: Artemisinin, *Plasmodium falciparum*, Microwave, Malaria

3.1 Introduction

Malaria is a protozoan infection transmitted to humans by the bite of an infected female anopheline mosquito. It is estimated that malaria kills about 655,000 people each year, 91% of whom are living in Africa and, most of them children under the age of 5 years.[1]

An increase in resistance against most of the drugs currently used against malaria has led to the dramatic increase in the global death toll. Chloroquine (CQ) and other synthetic quinoline compounds has been the mainstay of malaria chemotherapy for five decades, but resistance against these and the antifolate antimalarials have spread rapidly making these drugs obsolete in most of the areas afflicted by this disease.[2,3]

The artemisinin class of compounds (Fig. 1) are currently the basis of treatment preferred by the World Health Organization.[4] Artemisinin, a trioxane endoperoxide compound, is one of the most rapidly acting antimalarials with the broadest effective range but is however poorly soluble in both oil and water. In an effort to overcome this solubility problem, the first generation analogs of artemisinin, *viz*. artemether, arteether and sodium artesunate, were synthesised. Unfortunately these derivatives have relatively short elimination half-lives creating an elevated risk of high recrudescence rates.[5–7]

In an effort to prevent the artemisinin class of compounds from suffering the same fate as the classic antimalarial drugs, they are mostly used in artemisinin-based combination therapies (ACTs) which entail combining a semi-synthetic artemisinin derivative with another drug of a different chemical class. These ACTs do not only compensate for artemisinin's poor pharmacokinetic properties, but also decrease the likelihood that resistance against this class would emerge. Despite these efforts, resistance against artesunate has already been reported at the Thai-Cambodian and Thai-Myanmar borders, where significantly prolonged *in vivo* parasite clearance times have been observed.[8,9] This is a stark reminder of the exceptional ability of the malaria parasite to acquire resistance against a huge variety of chemical compounds, and a realisation that the loss of this important drug class would have dire consequences for millions of people around the world.

Polyamine compounds have been found to have implications in a great number of various processes in the malaria parasite. The natural occurring polyamines spermidine, putrescine and spermine have an important function in the regulation of growth and differentiation in an array of cell types.[10] These polyamine compounds, existing as polycations *in vivo*, are taken up by the malaria parasite through a polyamine transport system that recognises specific point charges on these compounds and then actively transports them into the malarial cells. The rapidly growing malaria parasite needs a substantial amount of these polyamine compounds and is reliant on exogenous sources of polyamines together with the polyamine transport system to provide it with the necessary quantities.[11]

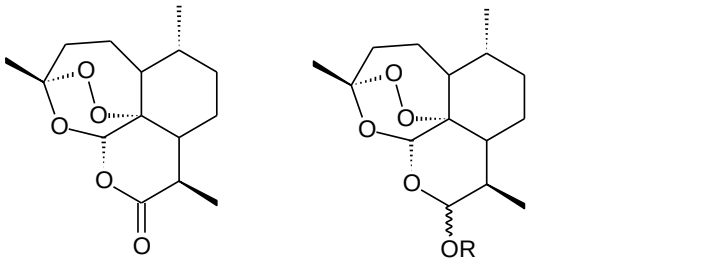


Figure 1: Artemisinin class of compounds

Researchers have proposed that a moiety that has the capability of being recognised by the polyamine transport system could act as a vector when coupled to another drug molecule, increasing the concentration and the potency of that given drug. Chadwick and co-workers[11] have attached spermidine and Boc-protected spermidine to artemisinin (ART) and have observed disappointing activity in the former but potent activity in the latter. They assigned the perceived disappointing activity to either poor membrane permeability of the ART-spermidine compounds, insufficient accumulation in the food vacuole of the parasite, or failure of the compounds to be recognised by the polyamine transport system.

Calas *et al.*[12] have proposed a second mechanism of action by which their primary, secondary, tertiary and quaternary amino compounds, with either one or two amino groups, had substantial activity against the malaria parasite. These compounds are analogues of choline, ethanolamine, serine and other polar head compounds that are essential for phospholipid biosynthesis, a process necessary for membrane synthesis in the malaria parasite. By competing with these naturally occurring polar head compounds, Calas *et al*.' compounds were able to block the synthesis of phospholipids, killing the parasite.[12,13] In their study, the length of the chain between the two amino groups, the degree of substitution and the number of lipophillic moieties around the amines had a substantial influence on the activity.[14]

The third mechanism of action suggested to explain the activity of amino compounds against malaria is ion trapping. The basis of this mechanism relies on the concept that amino groups in certain drugs, for example, chloroquine, act as weak bases. In the normal *in vivo* environment (pH 7.4), these amines are neutral and unprotonated thus having the capability to cross lipophillic membranes easily. However, when these unprotonated drug molecules reach the acidic (pH 4.5–5.5) food vacuole of the malaria parasite, they become protonated, making them less membrane permeable. This results in their accumulation in the digestive vacuole and consequently increases the potency of the given drug.[15] Previous studies

designed to increase the concentration of artemisinin by ion trapping resulted in a modest increase in antimalarial activity.[16,17]

The compounds described in this article are also amine derivatives of artemisinin and with different lipophillic moieties, various substituents and with varying chain lengths separating the amino groups. Such compounds may serve as possible drugs in the fight against malaria.

3.2 Materials and methods

3.2.1 Materials

Dihydroartemisinin (mixture of 10-α and 10-β epimers) was purchased from Changzhou Kaixuan Chemical Co (Chunjiang, China). Boron trifluoride diethyl etherate ($BF_3{\cdot}Et_2O$), 2-bromoethanol, N,N-dimethylformamide (DMF), morpholine, 4-(2-aminoethyl) morpholine, piperidine, 1-(2-aminoethyl)piperidine, 2-(diisopropylamino) ethylamine, N-benzylmethyl-amine, tetrahydrofuran (THF), 1-(3-aminopropyl)imidazole, N-phenylethylenediamine, piperazine and toluene were purchased from Sigma–Aldrich (Johannesburg, South Africa). Oxalic acid was purchased from BDH laboratory reagents (Yorkshire, England). Furfurylamine was purchased from Fluka (Johannesburg, South Africa). Isobutylamine was purchased from Merck (Johannesburg, South Africa). All the chemicals and reagents were of analytical grade. Chemicals were used without further purification.

3.2.2 General procedures

The ^1H and ^{13}C NMR spectra were recorded on a Bruker Avance™ III 600 spectrometer at a frequency of 600.17 and 150.913 MHz, respectively, in deuterated dimethyl sulfoxide (DMSO-d_6) or deuterated $CDCl_3$. Chemical shifts are reported in parts per million δ (ppm) with the residual protons of the solvent as reference. The splitting pattern abbreviations are as follows: s (singlet), d (doublet), dd (doublet of doublet), ddd (doublet of doublet of doublets), dt (doublet of triplets), dq (doublet of quartets), t (triplet), td (triplet of doublets), tt (triplet of triplets), qd (quartet of doublets), m (multiplet).

Mass spectra (MS) were recorded in positive mode on a Thermo Electron LXQ™ ion trap mass spectrometer. The MS had an APCI source set at 300 °C with Xcalibur 2.2 data acquisition and analysis software and utilised direct infusion with a Harvard syringe pump at a flow rate of 10 µL/min. A full scan from 100–1200 amu was obtained in 1 s, with a capillary voltage of 7 V while the corona discharge was 10 µA.

Melting points (Mp) were determined with a BÜCHI melting point B-545 instrument and were reported uncorrected. Thin layer chromatography was performed using silica gel plates ($60F_{254}$).

Microwave radiation was carried out using a CEM Discover™ focused closed vessel microwave synthesis system. The machine consists of a continuous focused microwave power delivery system with operator selectable power output from 0 to 300 W, a maximum current of 6.3 amps and a frequency of 50/60Hz. The temperature of the contents of the vessel was monitored using an IR sensor located underneath the reaction vessel. The contents of the vessel are stirred by means of a rotating magnetic plate located below the floor of the microwave cavity and a teflon coated magnetic stir bar in the vessel.

3.2.3 Biological testing

3.2.3.1 Antiplasmodial assay

The samples were tested in triplicate on one occasion against the chloroquine sensitive (CQS) strain of *Plasmodium falciparum* (D10), and on another occasion against the chloroquine resistant (CQR) strain of *Plasmodium falciparum* (Dd2). Continuous *in vitro* cultures of asexual erythrocyte stages of *P. falciparum* were maintained using a modified method of Trager and Jensen.[18] Quantitative assessment of antiplasmodial activity *in vitro* was determined *via* the parasite lactate dehydrogenase assay using a modified method.[19] The test samples were prepared to a 20 mg/ml stock solution in 100% DMSO and sonicated to enhance solubility. Samples were tested as a suspension if not completely dissolved. Stock solutions were stored at -20 °C. Further dilutions were prepared on the day of the experiment. Sodium artesunate (ArtNa), dihydroartemisinin (DHA) and chloroquine (CQ) were used as the reference drugs in all experiments. A full dose-response was performed for all compounds to determine the concentration inhibiting 50% of parasite growth (IC_{50} value). Samples were tested at a starting concentration of 100 ng/ml, which was then serially diluted 2-fold in complete medium to give 10 concentrations; with the lowest concentration being 0.2 ng/ml. The same dilution technique was used for all samples. The highest concentration of solvent to which the parasites were exposed to had no measurable effect on the parasite viability (data not shown). The IC_{50} values were obtained using a nonlinear dose-response curve fitting analysis via Graph Pad Prism v.4.0 software.

3.2.3.2 Cytotoxicity assay

The MTT-assay was used as a colorimetric assay for cellular growth and survival, and compares well with other available assays.[20,21] The tetrazolium salt MTT was used to measure all growth and chemosensitivity. The samples were tested in triplicate on one occasion against the mammalian cell-line, Chinese hamster ovarian (CHO). The same stock solutions used for antiplasmodial testing were used for cytotoxicity testing. Dilutions were

prepared on the day of the experiment. Emetine was used as the reference drug in all experiments. The initial concentration of emetine was 100 µg/ml, which was serially diluted in complete medium with 10-fold dilutions to give 6 concentrations, the lowest being 0.001 µg/ml. The same dilution technique was applied to all test samples. The highest concentration of solvent to which the cells were exposed to had no measurable effect on the cell viability (data not shown). The 50% inhibitory concentration (IC_{50}) values were obtained from full dose–response curves, using a non-linear dose–response curve fitting analysis via Graph Pad Prism v.4.0 software.

3.2.4 Statistical methods

3.2.4.1 Statistical analysis of antiplasmodial activity

One-way analyses of variances (ANOVA) were done to determine if significant differences existed between the means of the test compounds and each of artesunate, DHA and CQ in general. These were done for data on the D10 and Dd2 strains of *Plasmodium falciparum*. Levene's tests were performed to assure equality of variances in each ANOVA's case. In the case of inequality of variances Welch tests were performed. Normal probability plots on the residuals were done to assure that the data was fairly normally distributed.[22] Dunnett's tests were done to determine which of the test compounds' means differ statistically significantly from the mean of the standard compounds. Tukey's post hoc test was done to determine which of the test compounds' means differ statistically significantly from each other, omitting the reference drugs from the analysis. These procedures were done using the statistical data analysis software system.[23] All tests were done at a 0.05 significant level.

3.2.4.2 Statistical analysis of cytotoxicity

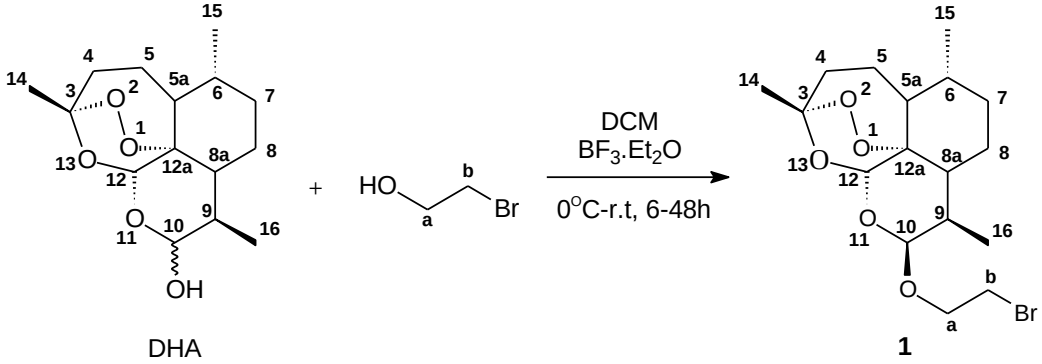
The statistical methods utilised to determine the cytotoxicity of the test compounds against CHO were similar to the methods used for antiplasmodial testing (see Section 3.2.4.1), using emetine as reference drug.

3.2.5 Synthesis

3.2.5.1 2-bromo-(10β-dihydroartemisinoxy) ethane, 1

The 1-bromo-2-(10β-dihydroartemisinoxy)ethane analogue **1** of DHA was synthesised by a method similar to that of Li and co-workers[24] (Scheme 1), which afforded **1** (76%) as a white powder.

M.p. 150.7°C; $C_{17}H_{27}BrO_5$. ^{1}H NMR (600 MHz, $CDCl_3$) δ 5.46 (s, 1H, H-12), 4.82 (d, *J* = 3.4 Hz, 1H, H-10), 4.13 (dt, *J* = 10.8, 5.4 Hz, 1H, H-aα), 3.76 (dt, *J* = 10.9, 5.3 Hz, 1H, H-aβ), 3.53 (t, 2H, H-b), 2.66 (dt, *J* = 7.7, 5.7 Hz, 1H, H-9), 2.33 (tt, *J* = 20.2, 10.0 Hz, 1H, H-4α), 2.04 – 1.97 (m, 1H, H-4β), 1.91 – 1.80 (m, 2H, H-8α, H-5α), 1.73 (ddd, *J* = 14.2, 7.5, 3.6 Hz, 1H, H-8β), 1.65 – 1.55 (m, 1H, H-7β), 1.52 – 1.37 (m, 5H, H-8a, H-5β, H-14), 1.36 – 1.28 (m, 1H, H-6), 1.25 – 1.17 (m, 1H, H-5a), 0.89 (m, 7H, H-7α, H-15, H-16). ^{13}C NMR (151 MHz, $CDCl_3$) δ 104.10 (C-3), 102.02 (C-10), 88.12 (C-12), 81.02 (C-12a), 68.15 (C-a), 52.55 (C-5a), 44.33 (C-8a), 37.37 (C-6), 36.37 (C-4), 34.63 (C-7), 31.41 (C-b), 30.83 (C-9), 26.12 (C-14), 24.62 (C-5), 24.34(C-8), 20.31(C-15), 12.91(C-16). COSY & HSQC (See annexure A). HRMS *m/z* $[M+H]^+$: 392.14 (Calcd. for $C_{21}H_{35}NO_6$: 391.130).



Scheme 1: Etheration of DHA using boron trifluoride etherate.

3.2.5.2 General procedure for the coupling of a primary/secondary amine to 1

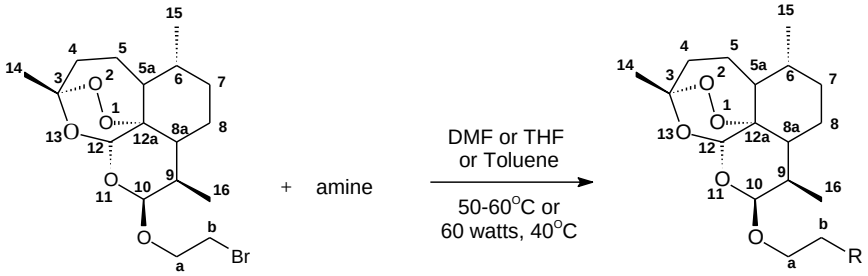
The pure compound **1** was dissolved in DMF, THF or toluene together with an excess of a given amine. The reaction mixture was either heated in an oil bath (50–60 °C) or a microwave reactor (in bursts of 60 watts and 40 °C for 4 minutes) until the reaction was complete (Scheme 2). After TLC showed the reaction to be completed it was stopped by firstly removing the solvent *in vacuo* and then extracting it with DCM and water. The DCM phase was then concentrated and the resulting oil was purified by column chromatography eluting with various ratios of MeOH, DCM, EtOAc and NH_4OH.

After purification the product was converted to the corresponding oxalate salt by stirring the obtained oil/powder with a solution of oxalic acid dissolved in diethyl ether (1 g/100ml). The resulting precipitate was filtered to deliver the product as a white/off white powder.

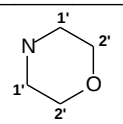
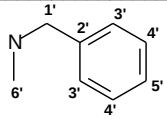
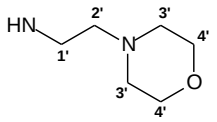
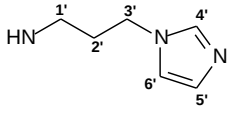
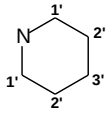
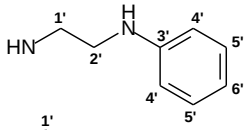
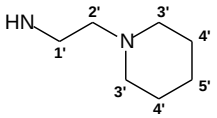
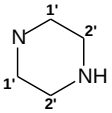
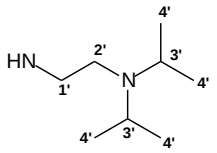
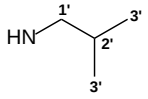
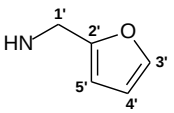
3.2.5.2.1 1-(morpholin-4-yl)-2-(10β-dihydroartemisinoxy)ethane oxalate, 2

Compound **1** (2.6 mmol, 1 g) was dissolved in 20 ml DMF and reacted with morpholine (10.2 mmol, 0.893 ml, 4 eq.) in an oil bath at 55 °C. Purification by column chromatography (silica gel, MeOH/DCM 1: 4) gave a white powder. The conversion into the oxalate salt

afforded 0.6 g (48%) of **2** as an off white powder, m.p. 171.0°C; $C_{21}H_{35}NO_6$. ^{1}H NMR (600 MHz, DMSO) δ 5.40 (s, 1H, H-12), 4.71 (d, *J* = 3.3 Hz, 1H, H-10), 3.93 – 3.85 (m, 1H, H-aα), 3.68 (d, *J* = 46.5 Hz, 4H, H-1'), 3.59 (dt, *J* = 10.4, 5.4 Hz, 1H, H-aβ), 3.07 – 2.80 (m, 6H, H-b, H-2'), 2.40 (dt, *J* = 7.8, 5.7 Hz, 1H, H-9), 2.17 (td, *J* = 14.1, 3.8 Hz, 1H, H-4α), 2.03 – 1.93 (m, 1H, H-4β), 1.86 – 1.76 (m, 1H, H-5α), 1.71 (qd, *J* = 13.5, 3.1 Hz, 1H, H-8α), 1.66 – 1.59 (m, 1H, H-8β), 1.54 (dd, *J* = 13.0, 3.0 Hz, 1H, H-7β), 1.42 – 1.30 (m, 3H, H-5β, H-6, H-8a), 1.27 (s, 3H, H-14), 1.13 (td, *J* = 11.4, 6.7 Hz, 1H, H-5a), 0.84 (ddd, *J* = 36.5, 21.4, 6.4 Hz, 7H, H-7α, H-15, H-16). ^{13}C NMR (151 MHz, DMSO) δ 103.44 (C-3), 100.91 (C-10), 87.07 (C-12), 80.55 (C-12a), 64.31 (C-1'), 62.89 (C-a), 56.17 (C-b), 52.32 (C-2'), 52.05 (C-5a), 43.66 (C-8a), 36.49 (C-6), 35.67 (C-4), 34.10 (C-7), 30.44 (C-9), 25.49 (C-14), 24.22 (C-5), 23.77 (C-8), 20.09 (C-15), 12.76 (C-16). COSY & HSQC (See annexure A). HRMS *m/z* $[M+H]^+$: 398.2545 (Calcd. for $C_{21}H_{35}NO_6$: 397.246438).



1 **2 - 12**

Compound	R	Compound	R
2		**8**	
3		**9**	
4		**10**	
5		**11**	
6		**12**	
7			

Scheme 2: Synthesis of aminoethylether derivatives of artemisinin.

3.2.5.2.2 1-{[2-(morpholin-4-yl)ethyl]amino}-2-(10β-dihydroartemisinoxy)ethane oxalate, 3

Compound **1** (1.3 mmol, 0.5 g) was dissolved in 3 ml DMF and reacted with 4-(2-aminoethyl)morpholine (5.1 mmol, 0.670 ml, 4 eq.) in a microwave reactor in bursts of 60 watts and 40°C for 4 minutes at a time. Purification by column chromatography (silica gel, MeOH) gave a white powder. The conversion into the oxalate salt afforded 0.4 g (70%) of **3** as an off white powder, m.p. 164.9°C; $C_{23}H_{40}N_2O_6$. ^{1}H NMR (600 MHz, $CDCl_3$) δ 5.37 (s, 1H, H-12), 4.78 (d, *J* = 3.2 Hz, 1H, H-10), 3.97 (dt, *J* = 10.7, 5.5 Hz, 1H, H-aα), 3.70 (m, 4H, H-3'), 3.48 (dt, *J* = 10.5, 5.3 Hz, 1H. H-aβ), 2.80 – 2.73 (m, 2H, H-b), 2.69 (t, *J* = 14.3, 8.3 Hz, 2H, H-1'), 2.60 (s, 1H, H-9), 2.50 – 2.44 (m, 2H, H-2'), 2.41 (s, 4H, H-4'), 2.32 (dt, *J* = 33.2, 19.8 Hz, 1H, H-4α), 2.03 – 1.90 (m, 1H, 4β), 1.83 (dt, *J* = 25.9, 13.3 Hz, 1H, H-5α), 1.78 – 1.66 (m, 2H, H-8β, H-8α), 1.59 (dd, *J* = 13.2, 3.1 Hz, 1H, H-7β), 1.51 – 1.36 (m, 5H, H-8a, H-5β, H-14), 1.30 (dd, *J* = 12.4, 8.6 Hz, 1H, H-6), 1.25 – 1.14 (m, 1H, H-5a), 0.96 – 0.68 (m, 7H, H-7α, H-15, H-16). ^{13}C NMR (151 MHz, $CDCl_3$) δ 104.06 (C-3), 102.07 (C-10), 87.83 (C-12), 81.00 (C-12a), 67.86 (C-a), 66.99 (C-3'), 58.35 (C-2'), 53.72 (C-5a), 52.50 (C-4'), 49.37 (C-b), 45.96 (C-1'), 44.28 (C-8a), 37.41 (C-6), 36.37 (C-4), 34.60 (C-7), 30.84 (C-9), 26.14 (C-14), 24.64 (C-5), 24.52 (C-8), 20.30 (C-15), 13.08 (C-16). COSY & HSQC (See annexure A). HRMS *m/z* $[M+H]^+$: 441.2958 (Calcd. for $C_{23}H_{40}N_2O_6$: 440.288637).

3.2.5.2.3 1-(piperidin-1-yl)-2-(10β-dihydroartemisinoxy)ethane oxalate, 4

Compound **1** (2.6 mmol, 1 g) was dissolved in 20 ml DMF and reacted with piperidine (10.2 mmol, 1.01 ml, 4 eq.) in an oil bath at 55 °C. Purification by column chromatography (silica gel, MeOH/DCM 1: 4) gave a white powder. The conversion into the oxalate salt afforded 0.9 g (76%) of **4** as an off white powder, m.p. 166.7°C; $C_{22}H_{37}NO_5$. ^{1}H NMR (600 MHz, DMSO) δ 5.39 (s, 1H, H-12), 4.72 (d, *J* = 3.4 Hz, 1H, H-10), 4.00 – 3.91 (m, 1H, H-aα), 3.69 – 3.58 (m, 1H,H-aβ), 3.16 (dd, *J* = 46.2, 20.7 Hz, 5H, H-b, H-1'), 2.45 – 2.36 (m, 1H, H-9), 2.17 (td, *J* = 14.1, 3.8 Hz, 1H, H-4α), 2.03 – 1.94 (m, 1H, H-4β), 1.85 – 1.76 (m, 1H, H-2', H-5α), 1.74 – 1.58 (m, 6H, H-2', H-8α, H-8β), 1.55 – 1.43 (m, 3H, H-3', H-7β), 1.35 (ddd, *J* = 18.2, 11.3, 5.0 Hz, 3H, H-5β, H-6, H-8a), 1.28 (s, 3H, H-14), 1.19 – 1.02 (m, 1H, H-5a), 0.99 – 0.63 (m, 7H, H-7α, H-15, H-16). ^{13}C NMR (151 MHz, DMSO) δ 103.39 (C-3), 100.78 (C-10), 87.00 (C-12), 80.47 (C-12a), 62.53 (C-a), 55.41 (C-b), 52.75 (C-1'), 52.03 (C-5a), 43.66 (C-8a), 36.48 (C-6), 35.82 (C-4), 34.15 (C-7), 30.28 (C-9), 25.49 (C-14), 24.22 (C-5), 23.91 (C-8), 22.63 (C-2'), 21.52 (C-3'), 20.14 (C-15), 12.69 (C-16). COSY & HSQC (See annexure A). HRMS *m/z* $[M+H]^+$: 396.2747 (Calcd. for $C_{22}H_{37}NO_5$: 395.267174).

3.2.5.2.4 1-{[2-(piperidin-1-yl)ethyl]amino}-2-(10β-dihydroartemisinoxy)ethane oxalate, 5

Compound **1** (1.3 mmol, 0.5 g) was dissolved in 3 ml DMF and reacted with 1-(2-aminoethyl)piperidine (5.1 mmol, 0.7257 ml, 4 eq.) in a microwave reactor in bursts of 60 watts and 40°C for 4 minutes at a time. Purification by column chromatography (silica gel, MeOH/DCM/NH_4 1: 18: 1) gave a white powder. The conversion into the oxalate salt afforded 0,2 g (23%) of **5** as an off white powder, m.p. 160.4°C; $C_{24}H_{42}N_2O_5$. ^{1}H NMR (600 MHz, DMSO) δ 5.39 (s, 1H, H-12), 4.71 (d, *J* = 3.3 Hz, 1H, H-10), 3.90 (dt, *J* = 10.4, 5.1 Hz, 1H, H-aα), 3.61 – 3.52 (m, 1H, H-aβ), 3.27 (d, *J* = 25.0 Hz, 2H, H-1'), 3.16 (d, *J* = 5.8 Hz, 2H, H-b), 3.04 (s, 2H, H-2'), 2.82 (d, *J* = 62.3 Hz, 4H, H-3'), 2.43 – 2.34 (m, 1H, H-9), 2.17 (td, *J* = 14.1, 3.8 Hz, 1H, H-4α), 2.02 – 1.94 (m, 1H, H-4β), 1.84 – 1.76 (m, 1H, H-5α), 1.67 (d, *J* = 24.9 Hz, 6H, H-8β, H-8α, H-4'), 1.57 – 1.50 (m, 1H, H-7β), 1.46 (s, 2H, H-5'), 1.41 – 1.31 (m, 3H, H-8a, H-6), 1.28 (s, 3H, H-14), 1.13 (td, *J* = 11.4, 6.7 Hz, 1H, H-5a), 0.94 – 0.76 (m, 7H, H-7α, H-15, H-16). ^{13}C NMR (151 MHz, DMSO) δ 103.35 (C-3), 101.06 (C-10), 86.99 (C-12), 80.49 (C-12a), 63.50 (C-a), 53.00 (C-3'), 52.73 (C-2'), 51.98 C-5a), 46.43 (C-b), 43.74 (C-8a), 42.34 (C-1'), 36.48 (C-6), 36.01 (C-4), 34.10 (C-7), 30.37 (C-9), 25.62 (C-14), 24.31 (C-4'), 23.90 (C-5'), 23.48 (C-5), 22.17 (C-8), 20.13 (C-15), 12.60 (C-16). COSY & HSQC (See annexure A). HRMS *m/z* $[M+H]^+$: 439.3166 (Calcd. for $C_{24}H_{42}N_2O_5$: 438.309373).

3.2.5.2.5 1-({2-[bis(propan-2-yl)amino]ethyl}amino)-2-(10β-dihydroartemisinoxy)ethane oxalate, 6

Compound **1** (2.6 mmol, 1 g) was dissolved in 15 ml DMF and reacted with 2-(diisopropylamino) ethylamine (12.8 mmol, 2.2 ml, 5 eq.) in a microwave reactor in bursts of 60 watts and 40°C for 4 minutes at a time. Purification by column chromatography (silica gel, MeOH/DCM/NH_4 1.5: 13: 0.5) gave a brown oil. The conversion into the oxalate salt afforded 0.5 g (33%) of **6** as a white powder, m.p. 139.4°C; $C_{25}H_{46}N_2O_5$. ^{1}H NMR (600 MHz, DMSO) δ 5.38 (s, 1H, H-12), 4.71 (s, 1H, H-10), 3.88 (s, 1H, H-aα), 3.54 (s, 1H, H-aβ), 3.12 – 3.00 (m, 4H, H-b, H-3'), 2.87 (d, *J* = 6.1 Hz, 2H, H-1'), 2.67 (s, 2H, H-2'), 2.36 - 2.45 (m, 1H, H-9), 2.17 (t, *J* = 13.5 Hz, 1H, H-4α), 1.99 (d, *J* = 13.8 Hz, 1H, H-4β), 1.79 (s, 1H, H-5α), 1.74 – 1.58 (m, 2H, H-8α, H-8β), 1.52 (d, *J* = 12.1 Hz, 1H, H-7β), 1.42 – 1.23 (m, 6H, H-5β, H-6, H-8a, H-14), 1.19 – 1.10 (m, 1H, H-5a), 1.02 – 0.80 (m, 21H, H-4', H-7α, H-15, H-16). ^{13}C NMR (151 MHz, DMSO) δ 103.44 (C-3), 101.03 (C-10), 86.90 (C-12), 80.34 (C-12a), 64.16 (C-a), 52.20 (C-5a), 47.31 (C-b), 46.78 (C-1'), 43.70 (C-8a), 41.25 (C-2'), 36.48 (C-6), 36.00 (C-4), 34.11 (C-7), 30.27 (C-9), 25.61 (C-14), 24.22 (C-5), 23.91 (C-8), 20.26 (C-4'), 20.10

(C-15), 12.62 (C-16). COSY & HSQC (See annexure A). HRMS *m/z* $[M+H]^+$: 455.3485 (Calcd. for $C_{25}H_{46}N_2O_5$: 454.340673).

3.2.5.2.6 1-[(furan-2-ylmethyl)amino]-2-(10β-dihydroartemisinoxy)ethane oxalate, 7

Compound **1** (2.6 mmol, 1 g) was dissolved in 15 ml DMF and reacted with furfurylamine (10.2 mmol, 0.95 ml, 4 eq.) in a microwave reactor in bursts of 60 watts and 40°C for 4 minutes at a time. Purification by column chromatography (silica gel, EtOAc) gave a brown oil. The conversion into the oxalate salt afforded 0.4 g (28%) of **7** as a white powder, m.p. 161.0°C; $C_{22}H_{33}NO_6$. ^{1}H NMR (600 MHz, DMSO) δ 7.75 (s, 1H, H-5'), 6.57 (s, 1H, H-4'), 6.52 (s, 1H, H-3'), 5.40 (s, 1H, H-12), 4.71 (s, 1H, H-10), 4.20 (s, 2H, H-1'), 3.89 (s, 1H, H-aα), 3.55 (s, 1H, H-aβ), 3.08 (s, 2H, H-b), 2.42 (s, 1H, H-9), 2.18 (s, 1H, H-4α), 2.01 (s, 1H, H-4β), 1.80 (s, 1H, H-5α), 1.66 (m, 2H, H-8α. H-8β), 1.54 (s, 1H, H-7β), 1.32 (m, 6H, H-6, H-8a, H-5β, H-14), 1.14 (s, 1H, H-5a), 0.88 (m, 7H, H-7α, H-15, H-16). ^{13}C NMR (151 MHz, DMSO) δ 146.84 (C-2'), 143.96 (C-5'), 111.41 (C-4'), 110.87 (C-3'), 103.43 (C-3), 101.06 (C-10), 86.89 (C-12), 80.34 (C-12a), 63.65 (C-a), 51.90 (C-5a), 45.75 (C-b), 43.83 (C-8a), 42.83 (C-1'), 36.46 (C-6), 36.01 (C-4), 34.09 (C-7), 30.27 (C-9), 25.62 (C-14), 24.22 (C-5), 23.95 (C-8), 20.11 (C-15), 12.62 (C-16). COSY & HSQC (See annexure A). HRMS *m/z* $[M+H]^+$: 408.2375 (Calcd. for $C_{22}H_{33}NO_6$: 407.230788).

3.2.5.2.7 1-[benzyl(methyl)amino]-2-(10β-dihydroartemisinoxy)ethane oxalate, 8

Compound **1** (2.6 mmol, 1 g) was dissolved in 20 ml THF and reacted with N-benzylmethylamine (10.2 mmol, 1.3 ml, 4 eq.) in an oil bath at 55 °C. Purification by column chromatography (silica gel, EtOAc) gave a colourless oil. The conversion into the oxalate salt afforded 0.6 g (43%) of **8** as an off white powder, m.p. 137.1°C; $C_{25}H_{37}NO_5$. ^{1}H NMR (600 MHz, DMSO) δ 7.50 – 7.32 (m, 5H, H-3',H-4', H-5'), 5.37 (s, 1H, H-12), 4.73 (d, *J* = 3.3 Hz, 1H, H-10), 4.09 (d, *J* = 29.8 Hz, 2H, H-1'), 3.98 – 3.87 (m, 1H, H-aα), 3.67 – 3.58 (m, 1H, H-aβ), 3.08 (s, 2H, H-b), 2.51 (s, 3H, H-6'), 2.40 (dt, *J* = 14.9, 5.7 Hz, 1H, H-9), 2.17 (td, *J* = 14.1, 3.8 Hz, 1H, H-4α), 2.02 – 1.93 (m, 1H, H-4β), 1.84 – 1.76 (m, 1H, H-5α), 1.74 – 1.57 (m, 2H, H-8α, H-8β), 1.52 (dd, *J* = 13.0, 2.8 Hz, 1H, H-7β), 1.40 – 1.23 (m, 6H, H-5β, H-6, H-8a, H-14), 1.18 – 1.04 (m, 1H, H-5a), 0.95 – 0.76 (m, 8H, H-7α, H-15, H-16). ^{13}C NMR (151 MHz, DMSO) δ 130.27 (C-2'), 128.53 (C-3'), 128.38 (C-4'), 128.23 (C-5'), 103.29 (C-3), 100.87 (C-10), 86.99 (C-12), 80.45 (C-12a), 63.34 (C-a), 59.75 (C-1'), 55.02 (C-b), 51.88 (C-5a), 43.65 (C-8a), 40.48 (C-6'), 36.52 (C-6), 35.78 (C-4), 33.99 (C-7), 30.20(C-9), 25.54 (C-14), 24.20 (C-5), 23.76 (C-8), 19.97 (C-15), 12.54 (C-16). COSY & HSQC (See annexure A). HRMS *m/z* $[M+H]^+$: 432.2746 (Calcd. for $C_{25}H_{37}NO_5$: 431.267174).

3.2.5.2.8 1-{[3-(1H-imidazol-1-yl)propyl]amino}-2-(10β-dihydroartemisinoxy)ethane oxalate, 9

Compound **1** (1.3 mmol, 0.5 g) was dissolved in 3 ml DMF and reacted with 1-(3-aminopropyl)imidazole (6.4 mmol, 0.766 ml, 5 eq.) in a microwave reactor in bursts of 60 watts and 40°C for 4 minutes at a time. Purification by column chromatography (silica gel, MeOH/DCM/NH_4 1: 13: 1) gave a yellow oil. The conversion into the oxalate salt afforded 0.3 g (44%) of **9** as a off white powder, m.p. 148.2°C; $C_{23}H_{37}N_3O_5$. ^{1}H NMR (600 MHz, DMSO) δ 8.16 (d, *J* = 13.3 Hz, 1H, H-6'), 7.37 (s, 1H, H-5'), 7.16 (s, 1H, H-4'), 5.38 (s, 1H, H-12), 4.69 (d, *J* = 3.4 Hz, 1H, H-10), 4.13 (t, *J* = 6.8 Hz, 2H, H-1'), 3.89 (dt, *J* = 10.8, 5.3 Hz, 1H, H-aα), 3.61 – 3.47 (m, 1H, H-aβ), 3.19 – 3.05 (m, 2H, H-b), 2.93 – 2.81 (m, 2H, H-3'), 2.41 – 2.32 (m, 1H, H-9), 2.22 – 2.03 (m, 3H,H-4α, H-2'), 2.02 – 1.92 (m, 1H, H-4β), 1.82 – 1.74 (m, 1H, H-5α), 1.69 – 1.54 (m, 2H, H-8β, H-8α), 1.52 (dd, *J* = 12.9, 2.9 Hz, 1H, H-7β), 1.43 – 1.25 (m, 6H, H-5β, H-6, H-8a, H-14), 1.15 – 1.00 (m, 1H, H-5a), 0.83 (ddd, *J* = 26.8, 18.5, 6.4 Hz, 8H, H-7α, H-15, H-16). ^{13}C NMR (151 MHz, DMSO) δ 136.66 (C-6'), 125.69 (C-4'), 120.12 (C-5'), 103.43 (C-3), 100.90 (C-10), 86.90 (C-12), 80.33 (C-12a), 63.34 (C-a), 52.05 (C-5a), 45.85 (C-b), 44.25 (C-3'), 43.96 (C-1'), 43.66 (C-8a), 36.33 (C-6), 36.01 (C-4), 34.10 (C-7), 30.27 (C-9), 26.90 (C-2'), 25.62 (C-14), 24.21 (C-5), 23.76 (C-8), 20.12 (C-15), 12.62 (C-16). COSY & HSQC (See annexure A). HRMS *m/z* $[M+H]^+$: 436.2807 (Calcd. for $C_{23}H_{37}N_3O_5$: 435.273322).

3.2.5.2.9 1-{[2-(phenylamino)ethyl]amino}-2-(10β-dihydroartemisinoxy)ethane oxalate, 10

Compound **1** (2.6 mmol, 1 g) was dissolved in 15 ml DMF and reacted with N-phenylethylenediamine (12.8 mmol, 1.7 ml, 5 eq.) in a microwave reactor in bursts of 60 watts and 40°C for 4 minutes at a time. Purification by column chromatography (silica gel, MeOH) gave a brown oil. The conversion into the oxalate salt afforded 1.0 g (74%) of **10** as an off white powder, m.p. 151.6°C; $C_{25}H_{38}N_2O_5$. ^{1}H NMR (600 MHz, DMSO) δ 7.08 (t, *J* = 7.8 Hz, 2H, H-4'), 6.65 – 6.51 (m, 3H, H-5', H6'), 5.39 (s, 1H, H-12), 4.71 (d, *J* = 3.4 Hz, 1H, H-10), 3.92 (dt, *J* = 10.7, 5.2 Hz, 1H, H-aα), 3.62 – 3.53 (m, 1H, H-aβ), 3.34 (dd, *J* = 16.1, 9.6 Hz, 2H, H-2'), 3.23 – 3.15 (m, 2H, H-b), 3.13 – 3.05 (m, 2H, H-1'), 2.43 – 2.34 (m, 1H, H-9), 2.17 (td, *J* = 14.1, 3.8 Hz, 1H, H-4α), 2.04 – 1.93 (m, 1H, H-4β), 1.84 – 1.74 (m, 1H, H-5α), 1.72 – 1.54 (m, 2H, H-8α, H-8β), 1.49 (dd, *J* = 13.0, 3.0 Hz, 1H, H-7β), 1.39 – 1.19 (m, 6H, H-6, H-8a, H-5β, H-14), 1.19 – 1.04 (m, 1H, H-5a), 0.82 (ddd, *J* = 16.2, 13.6, 5.1 Hz, 7H, H-7α, H-15, H-16). ^{13}C NMR (151 MHz, DMSO) δ 147.97 (C-3'), 128.89 (C-4'), 116.32 (C-6'), 112.34 (C-5'), 103.43 (C-3), 101.03 (C-10), 86.91 (C-12), 80.35 (C-12a), 63.50 (C-a), 52.02

(C-5a), 46.21 (C-1'), 46.02 (C-b), 43.66 (C-8a), 41.15 (C-2'), 36.33 (C-6), 35.81 (C-4), 34.11 (C-7), 30.27 (C-9), 25.49 (C-14), 24.23 (C-5), 23.89 (C-8), 20.12 (C-15), 12.62 (C-16). COSY & HSQC (See annexure A). HRMS *m/z* $[M+H]^+$: 447.2858 (Calcd. for $C_{25}H_{38}N_2O_5$: 446.278073).

3.2.5.2.10 1-(piperazin-1-yl) -2-(10β-dihydroartemisinoxy)ethane oxalate, 11

Compound **1** (2.6 mmol, 1 g) was dissolved in 3 ml toluene and reacted with piperazine (12.8 mmol, 1 g, 5 eq.) in a microwave reactor in bursts of 60 watts and 40°C for 4 minutes at a time. Purification by column chromatography (silica gel, MeOH/DCM/NH_4 1: 9: 0.5) gave a brown oil. The conversion into the oxalate salt afforded 0.7 g (53%) of **11** as a white powder, m.p. 159.23°C; $C_{21}H_{36}N_2O_5$. ^{1}H NMR (600 MHz, DMSO) δ 5.38 (s, 1H, H-12), 4.68 (d, *J* = 2.9 Hz, 1H, H-10), 3.78 – 3.70 (m, 1H, H-aα), 3.46 (dt, *J* = 10.5, 5.3 Hz, 1H, H-aβ), 3.04 (s, 4H, H-2'), 2.71 – 2.52 (m, 6H, H-b, H-1'), 2.37 (d, *J* = 3.2 Hz, 1H, H-9), 2.17 (td, *J* = 14.1, 3.5 Hz, 1H, H-4α), 1.98 (d, *J* = 14.2 Hz, 1H,H-4α), 1.84 – 1.67 (m, 2H, H-8α, H-5α), 1.65 – 1.57 (m, 1H, H-8β), 1.56 – 1.50 (m, 1H, H-7β), 1.40 – 1.19 (m, 6H, H-5β, H-6, H-8a, H-14), 1.13 (dt, *J* = 17.9, 9.0 Hz, 1H, H-5a), 0.85 (dd, *J* = 35.6, 6.7 Hz, 7H, H-7α, H-15, H-16). ^{13}C NMR (151 MHz, DMSO) δ 103.36 (C-3), 100.79 (C-10), 86.89 (C-12), 80.49 (C-12a), 64.92 (C-a), 56.90 (C-b), 52.10 (C-5a), 49.55 (C-1'), 43.86 (C-2'), 42.87 (C-8a), 36.82 (C-6), 36.07 (C-4), 34.16 (C-7), 30.48 (C-9), 25.68 (C-14), 24.34 (C-5), 23.97 (C-8), 20.22 (C-15), 12.81 (C-16). COSY & HSQC (See annexure A). HRMS *m/z* $[M+H]^+$: 397.2699 (Calcd. for $C_{21}H_{36}N_2O_5$: 396.262423).

3.2.5.2.11 1-[(2-methylpropyl)amino] -2-(10β-dihydroartemisinoxy)ethane oxalate, 12

Compound **1** (2.6 mmol, 1 g) was dissolved in 15 ml DMF and reacted with isobutylamine (10.2 mmol, 1.0 ml, 4 eq.) in a microwave reactor in bursts of 60 watts and 40°C for 4 minutes at a time. Purification by column chromatography (silica gel, MeOH) gave a colourless oil. The conversion into the oxalate salt afforded 0.4 g (23%) of **12** as a white powder, m.p. 161.7°C; $C_{21}H_{37}NO_5$. ^{1}H NMR (600 MHz, DMSO) δ 5.40 (s, 1H, H-12), 4.71 (t, *J* = 5.9 Hz, 1H, H-10), 3.97 – 3.88 (m, 4H, H-aα), 3.57 (dd, *J* = 11.0, 5.6 Hz, 1H, H-aβ), 3.20 – 3.05 (m, 2H, H-b), 2.88 – 2.68 (m, 2H, H-1'), 2.41 (dd, *J* = 7.1, 3.8 Hz, 1H, H-9), 2.17 (td, *J* = 14.1, 3.6 Hz, 1H, 4α), 2.06 – 1.84 (m, 2H, H-4β, H-2'), 1.83 – 1.73 (m, 1H, 5α), 1.72 – 1.57 (m, 2H, H-8α, H-8β), 1.53 (d, *J* = 10.3 Hz, 1H, H-7β), 1.44 – 1.17 (m, 6H, H-5β, H-6, H-8a, H-14), 1.19 – 1.09 (m, 1H, H-5a), 0.98 – 0.61 (m, 15H, H-3', 7α, H-15, H-16). ^{13}C NMR (151 MHz, DMSO) δ 103.24 (C-3), 101.06 (C-10), 87.07 (C-12), 80.35 (C-12a), 63.36 (C-a), 54.32 (C-2'), 51.75 (C-5a), 46.36 (C-b), 43.68 (C-8a), 36.47 (C-6), 36.00 (C-4), 34.09 (C-7), 30.11

(C-9), 25.62 (C-1'), 25.36 (C-14), 24.37 (C-5), 23.93 (C-8), 20.12 (C-15), 19.94 (C-3'), 12.63 (C-16). COSY & HSQC (See annexure A). HRMS *m/z* $[M+H]^+$: 384.2745 (Calcd. for $C_{21}H_{37}NO_5$: 383.267174).

3.3 Results

3.3.1 Chemistry

Condensation of dihydroartemisinin with 2-bromoethanol produced intermediate **1** in good yield (Scheme 1).[24] This intermediate and all the other products were in the 10β form, as is confirmed by a coupling constant of J = 3.4 Hz between H-10 and H-9, which is consistent with a *cis*-equatorialaxial arrangement of these protons in a chair pyranose ring[25] and was previously determined by X-ray analysis.[26] A given amine was then reacted with **1** using either the described method with conventional heating, or for the first time with microwave radiation (Scheme 2) rendering products **2** to **12**. Compounds **2** and **11** have already been synthesised by Li and co-workers using the conventional heating method.[24]

3.3.2 *In vitro* antimalarial activity and cytotoxicity

The antimalarial activity and cytotoxicity of the synthesised compounds are given in Tables 1 and 2. All of the compounds were tested as oxalic salts and in the 10β-isomer form. Comparing the antimalarial activity of the compounds to that of artesunate, Dunnett's test shows that the mean IC_{50} values of compounds **2**–**7** and **9**–**12** differ statistically significantly from the mean of artesunate since they have *p*-values smaller than 0.05, implying that these compounds were less potent than artesunate against the D10 strain. The IC_{50} value of compound **8**, however, had a *p*-value greater than 0.05 indicating that there is no statistically significant difference from the mean value of artesunate implying that this compound was as potent against the D10 strain as artesunate. Against the Dd2 strain, however, derivatives **3**, **5**, **9**, **10** and **12** were less potent whilst **2**, **4**, **6**-**8**, and **11** all displayed potency similar to that of artesunate (Table 1).

In comparison with DHA, all the derivatives were found to be less potent against the D10 strain. Compounds **2**, **7** and **8**, however, had mean IC_{50} values against the Dd2 strain that were not statistically significantly different from that of DHA, implying that these compounds were equipotent to DHA (Table 1).

Compared to chloroquine all derivatives were more potent against both the D10 and Dd2 strains, with the exception of **9** that was as potent as CQ against the Dd2 strain.

The very low cytotoxicity of compounds **2**–**6**, **9** and **11** is illustrated by their IC_{50} values being above the maximum concentration used in this test. Dunnett's test showed that the

cytotoxicity of compounds **7**, **8**, **10** and **12** differed statistically significantly from that of emetine indicating that these compounds were less toxic than emetine (Table 2).

Table 1. Descriptive statistics of antimalarial IC_{50} values, results of ANOVAs and Dunnett's tests of compounds **2**-**12**, artesunate, DHA and chloroquine.

Compound	D10							Dd2							RI[b]
	n[a]	Mean IC_{50} (nM)	Std. dev.	*p*-value: Welch	*p*-value: Dunnett			n[a]	Mean IC_{50} (nM)	Std. dev.	*p*-value: Welch	*p*-value: Dunnett			
					ArtNa	DHA	CQ					ArtNa	DHA	CQ	
2	3	4.637	0.077		0.000*	0.000*	0.000*	3	16.151	1.681		0.920	0.743	0.000*	3.483
3	3	6.885	0.331		0.000*	0.000*	0.000*	3	61.585	4.659		0.008*	0.000*	0.000*	8.945
4	3	3.312	0.067		0.000*	0.000*	0.000*	3	32.109	0.000		0.995	0.043*	0.000*	9.695
5	3	6.718	0.369		0.000*	0.000*	0.000*	3	66.727	6.726		0.002*	0.000*	0.000*	9.932
6	3	1.833	0.046		0.001*	0.000*	0.000*	3	48.096	4.140		0.171	0.000*	0.000*	26.240
7	3	2.413	0.111		0.000*	0.000*	0.000*	3	21.374	1.775		1.000	0.374	0.000*	8.858
8	3	1.444	0.058		0.114	0.023*	0.000*	3	20.059	0.883		0.998	0.459	0.000*	13.888
9	3	6.038	0.419		0.000*	0.000*	0.000*	3	189.258	38.270		0.000*	0.000*	0.219	31.343
10	3	2.612	0.340		0.000*	0.000*	0.000*	3	55.085	2.937		0.038*	0.000*	0.000*	21.086
11	3	1.799	0.279		0.002*	0.000*	0.000*	3	50.943	5.063		0.095	0.000*	0.000*	28.318
12	3	4.015	0.213		0.000*	0.000*	0.000*	3	54.930	3.559		0.039*	0.000*	0.000*	13.680
ArtNa	3	0.933	0.161	0.000*				3	25.854	0.511	0.000*				27.719
DHA	3	0.785	0.142	0.000*				3	3.482	0.325	0.000*				4.433
CQ[†]	4	46.644	2.348	0.000*				4	168.740	12.656	0.000*				3.618

[*] Statistically significant at 0.05 level. Note, values < 0.05 indicates a statistically significant difference between the mean IC_{50} values with more than 95% certainty. [a] Replicates (n). [b] Resistance index (Dd2/D10). [†] CQ was tested as diphosphate salt.

Table 2. Descriptive statistics of cytotoxicity IC_{50} values, results of ANOVA and Dunnett's test of compounds **7**, **8**, **10**, **12** and emetine.

Compound	CHO				
	n[a]	Mean IC_{50} (nM)	Std. dev.	*p*-value: Welch	*p*-value: Dunnett
					Emetine
7	3	79672.800	4560.281		0.002*
8	3	140958.200	31708.460		0.000*
10	3	47471.900	14112.55		0.042*
12	3	72921.010	8151.421		0.004*
Emetine	2	19.765	10.298	0.000*	

* Statistically significant at 0.05 level. Note, values < 0.05 indicates a statistically significant difference between the mean IC_{50} values with more than 95% certainty. [a] Replicates (n). Note that the values of compounds **2**-**6**, **9** and **11** were above the maximum concentration used in this test and are therefore not included in the statistical analysis.

3.4 Discussion

3.4.1 Chemistry

The amino moieties were attached to DHA through an ethyl ether linker. This ether bond is notoriously labile and is rapidly metabolised by human liver microsomes.[27] Artemether, an ether derivative of artemisinin, is metabolised *in vivo* reproducing DHA within two hours after intramuscular administration.[28] The synthesised compounds **2**–**12** were, however, tested *in vitro* which preserves the ether bond and allows the compounds to act as derivatives and not as prodrugs.

Compounds **3**, **5**, **6**, **7**, **9**, **10**, **11** and **12** were synthesised using a microwave reactor. This is to our knowledge the first time that artemisinin compounds were subjected to microwave radiation. Special precautions were taken to assure that the peroxide bond remained intact during this procedure as this functional group is reported to be susceptible to facile hydrolysis under basic and acidic conditions[29] and can also be expected to be sensitive to heat. We found the peroxide bridge to be stable and were able to heat the reaction to more than 90 °C

without cleavage of the peroxide bond. This perceived stability was confirmed by the literature where the peroxide bond proved to be stable, even toward some highly reactive organometallic reagents.[30] Increasing either the duration or the power of the radiation led to the formation of a large number of by-products, most probably as result of the cleavage of the endoperoxide bridge.[29] Microwave radiation decreased the overall reaction time, but had no noticeable influence on the yield of the reactions. This can be seen when looking at compound **11** that had a yield of 53% in this method, compared to the reported 60%.[24] ^{13}C NMR spectrometry and high resolution MS confirmed conclusively that the peroxide bridge was preserved during microwave radiation in that no deviations from the expected values were observed in the spectra of all prepared compounds.

For stability and solubility reasons, the free base target compounds were converted into oxalate salts.

The structures of all synthesised compounds were confirmed by HRMS, ^{1}H and ^{13}C NMR techniques. Due to the rigid structure and asymmetric carbon units in the artemisinin nucleus, the methylene protons in positions 4, 5, 7 and 8 are all non-equivalent to one another and in each case appear as an AB pattern at δ 2.17 (4α), 2.06 (4β), 1.83 (5α), 1.44 (5β), 1.53 (7α), 0.98 (7β), 1.72 (8α and 8β), respectively. H-6, H-5a, H-8a and H-9, integrating for one proton each, gave signals at δ 1.44, 1.19, 1.17 and 2.41, respectively. The above mentioned signals together with the characteristic signals of H-12, H-10 and the methyl signals of H-14, H-15 and H-16, at δ 5.40, 4.71, 1.44, 0.98 and 0.61, respectively, confirmed the presence of the artemisinin moiety in the structures of all synthesised compounds. The protons H-a of the ether linker are also non-equivalent, appearing in a characteristic AB pattern at δ 3.9 (aα) and 3.5 (aβ), and serves as a marker that confirms the linker was still intact during the second step of the synthetic process. The most characteristic ^{13}C NMR signals of artemisinin are those of C-10, C-12, C-3 and C-12a at δ 100.90, 86.90, 103.43 and 80.33, respectively. The signals of the methyl carbon atoms C-14, C-15 and C-16 can also clearly be seen at δ 25.62, 20.12 and 12.62, respectively. The signal of the linker's carbon atom C-a can usually be found at d 63.34.

3.4.2 *In vitro* antimalarial activity and cytotoxicity

All the synthesised compounds were more potent than CQ against the chloroquine sensitive (CQS) D10 strain. Against the chloroquine resistant (CQR) Dd2 strain, they showed better activity than CQ with only compound **9** being as active as CQ. Of all the synthesised compounds, compound **8** (IC_{50} = 1.44 nM) had the highest activity against the D10 strain with activity comparable to that of artesunate. Against the Dd2 strain compounds **2**, **4**, **6**–**8** and **11**

were as active as artesunate, whilst compounds **2**, **7** and **8** had activity comparable to that of the even more potent DHA.

An interesting observation in this study was that compounds with shorter chain lengths displayed higher activity than those with longer chains connecting the DHA and amine moieties. An increase in chain length from two to five methylene equivalent units led to a reduction in activity as can be seen from the difference in activity of **2** (IC_{50} = 4.64 nM) versus that of **3** (6.89 nM) and **4** (3.31 nM) versus **5** (6.72 nM) for the D10 strain, and **2** (16.15 nM) versus **3** (61.59 nM) and **4** (32.11 nM) versus **5** (66.73 nM) for the Dd2 strain. For both strains Tukey's test confirms that the mean IC_{50} values of **2** and **3**, and that of **4** and **5** differ statistically significantly from one another. While the difference in activity can be specified with 95% certainty for both pairs against the D10 strain, against the Dd2 strain the difference in activity for latter pair can only be specified with 94% certainty, as can be seen from the *p*-value of 0.057 (Tables 3 and 4). Calas et al.[12] in their series of amino compounds found that those with longer chain lengths displayed better activity than those with shorter chains. This observation might suggest that our compounds perhaps do not share the same mechanism of action, having too great a difference in their pharmacophore.

Compounds **2**, **4** and **11** forms a homologous series in that they differ from one another in respect to the cyclic amino substituent, with **2** having an oxygen atom in position 4, **4** a methylene group and **11**, a NH group. Tukey's test confirms that the mean IC_{50} values of **2**, **4** and **11** differ statistically significantly from one another against the D10 strain.

The calculated pK_a value of compound **2** is 6.75, followed by that of **4** at 8.81 and then that of **11** at 9.31.[31] Their pK_a values are thus in the order **11** > **4** > **2**. On the basis of their antimalarial activity against the D10 strain, these compounds can also be arranged in the order **11** > **4** > **2**, from most to least active. Similarly the order of the resistance index (RI) values of these three compounds is **11** > **4** > **2**, which is also the same order as the pK_a values showing that the compound with the lowest pK_a value has the lowest discrimination between the two strains. A lower pK_a value of the tertiary amine implies a weaker base and this decreases the likelihood of the compound to be trapped in the parasite's acidic food vacuole *via* ion trapping. Whether there is any correlation between the difference in activity against the two strains, the pK_a values of the amine moieties and the mode by which the parasite acquires resistance warrants further investigation.

Short chain compounds with fewer amino groups, that is, **6**–**8** and **11** had better activity than the long chain polyamines, that is **3**, **5** and **9** against the D10 strain while the same observation was made comparing **7** and **8** to **3**, **5** and **9** against the Dd2 strain.

Table 3. *p*-Values of Tukey's test for compounds **2** – **12** on the D10 strain.

Compound		**2**	**3**	**4**	**5**	**6**	**7**	**8**	**9**	**10**	**11**	**12**
	Mean IC_{50} (nM)	4.637	6.885	3.312	6.718	1.833	2.413	1.444	6.038	2.612	1.799	4.015
2	4.637		0.000*	0.000*	0.000*	0.000*	0.000*	0.000*	0.000*	0.000*	0.000*	0.143
3	6.885	0.000*		0.000*	0.999	0.000*	0.000*	0.000*	0.015*	0.000*	0.000*	0.000*
4	3.312	0.000*	0.000*		0.000*	0.000*	0.008*	0.000*	0.000*	0.068	0.000*	0.065
5	6.718	0.000*	0.999	0.000*		0.000*	0.000*	0.000*	0.082	0.000*	0.000*	0.000*
6	1.833	0.000*	0.000*	0.000*	0.000*		0.206	0.709	0.000*	0.030*	1.000	0.000*
7	2.413	0.000*	0.000*	0.008*	0.000*	0.206		0.004*	0.000*	0.995	0.153	0.000*
8	1.444	0.000*	0.000*	0.000*	0.000*	0.709	0.004*		0.000*	0.000*	0.802	0.000*
9	6.038	0.000*	0.015*	0.000*	0.082	0.000*	0.000*	0.000*		0.000*	0.000*	0.000*
10	2.612	0.000*	0.000*	0.068	0.000*	0.030*	0.995	0.000*	0.000*		0.021*	0.000*
11	1.799	0.000*	0.000*	0.000*	0.000*	1.000	0.153	0.802	0.000*	0.021*		0.000*
12	4.015	0.143	0.000*	0.065	0.000*	0.000*	0.000*	0.000*	0.000*	0.000*	0.000*	

* Statistically significant at 0.05 level. Note, values < 0.05 indicates a statistically significant difference between the mean IC_{50} values with more than 95% certainty.

Table 4. *p*-Values of Tukey's test for compounds **2** – **12** on the Dd2 strain

Compound		**2**	**3**	**4**	**5**	**6**	**7**	**8**	**9**	**10**	**11**	**12**
	Mean IC_{50} (nM)	16.151	61.585	32.109	66.727	48.096	21.374	20.059	189.258	55.085	50.943	54.930
2	16.151		0.005*	0.858	0.002*	0.098	1.000	1.000	0.000*	0.022*	0.055	0.023*
3	61.585	0.001*		0.158	1.000	0.944	0.017*	0.013*	0.000*	1.000	0.989	1.000
4	32.109	0.858	0.158		0.057**	0.857	0.988	0.973	0.000*	0.448	0.705	0.457
5	66.727	0.002*	1.000	0.057**		0.717	0.005*	0.004*	0.000*	0.978	0.866	0.976
6	48.096	0.098	0.944	0.857	0.717		0.256	0.205	0.000*	1.000	1.000	1.000
7	21.374	1.000	0.017*	0.988	0.005*	0.256		1.000	0.000*	0.069	0.155	0.071
8	20.059	1.000	0.013*	0.973	0.004*	0.205	1.000		0.000*	0.052	0.121	0.054
9	189.258	0.000*	0.000*	0.000*	0.000*	0.000*	0.000*	0.000*		0.000*	0.000*	0.000*
10	55.085	0.022*	1.000	0.448	0.978	1.000	0.069	0.052	0.000*		1.000	1.000
11	50.943	0.055	0.988	0.705	0.866	1.000	0.155	0.121	0.000*	1.000		1.000
12	54.930	0.023*	1.000	0.457	0.976	1.000	0.071	0.054	0.000*	1.000	1.000	

* Statistically significant at 0.05 level. Note, values < 0.05 indicates a statistically significant difference between the mean IC_{50} values with more than 95% certainty. ** Statistically significant at 0.06 level.

A possible reason for this phenomenon could be that the longer chain polyamino compounds are too polar to cross the parasitic membranes while their short chain counterparts were lipophillic enough to reach the site of action. Interestingly, the difference in activity of the long chain polyamino compounds **6**, **9**–**11** against the resistant and sensitive strains as displayed by their RI values, is greater than for the short chain compounds. Whether this is due to the specific method by which the parasite acquires resistance, for example targeting polyamine compounds, still needs to be elucidated.

Compound **2**, with a short chain and one amino group, had the lowest IC_{50} value of all the synthesised compounds against the Dd2 strain. This observation correlates well with the above findings that short chain mono amino compounds retain a higher degree of activity than the longer chain polyamino compounds. Compound **8** with good activity against both the D10 and Dd2 strains features a tertiary amino group and an aryl ring. Compound **9** with a side chain containing a secondary amine and a terminal imidazole ring was the compound with the most nitrogen atoms and had the lowest overall activity, especially against the Dd2 strain (IC_{50} = 189.26 nM), and the highest RI value (RI = 31.34).

Against both strains, none of the compounds had activity higher than that of DHA or artesunate. While the addition of amino moieties to artemisinin had some favourable consequences, other factors such as H bonding, molecular shape, polarity, flexibility and charge/ionization also influence the accumulation of the drug at the site of action thus increasing or decreasing the activity of the drug. With DHA and artesunate having largely the same structure as the synthesized compounds, it is not unexpected they might exert their antiplasmodial effect to roughly the same extend.[32]

The conclusions drawn on the activity of compounds **2**–**12** are supported by statistical analysis of the data generated by the antimalarial test, but no attempts were made to draw any structure activity relationships as the set of compounds (11 in total) does not allow such an endeavour.

All of the synthesised compounds had a high degree of selectivity towards the parasite, with toxicity against CHO cells statistically significantly below that of the reference drug, emetine.

3.5 Conclusion

In this study eleven 10-aminoethylether derivatives of artemisinin were successfully synthesised by attachment of various amino moieties through an ether linkage to C-10 of DHA. The structures of the products were confirmed by NMR and

HRMS. The antimalarial activity of the compounds against both the D10 and Dd2 strains of *Plasmodium falciparum* were determined with CQ, sodium artesunate and DHA as references. All compounds were found to be more potent than CQ against either strain, with only compound **9** being equally as active against the Dd2 strain. Derivative **8** was as potent as artesunate against the D10 strain while compounds **2**, **4**, **6**–**8** and **11** had potency similar to that of artesunate against the Dd2 strain. No compound possessed higher or comparable activity to DHA against the D10 strain. However, compounds **2**, **7** and **8** were found as potent as DHA against the Dd2 strain.

It was observed in a small group of three homologous compounds that the order of the IC_{50} values against the D10 strain was the same as the order of their pK_a values as was the order of their RI values.

The short chain compounds were more active than the long chain polyamine containing compounds irrespective of the strain.

In summary, this study delivered three compounds, viz., **2**, **7** and **8**, that were as potent as DHA against the chloroquine resistant Dd2 stain, and one derivative, viz., **8**, that was as potent as artesunate against the D10 strain. Current efforts are underway to determine *the in vivo* IC_{50} values of these compounds and to elucidate their pharmacokinetic properties. They stand as good drug candidates to be further investigated in search for potent new antimalarial drugs.

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Supplementary data

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