Chapter 5

Synthesis, antimalarial activity and cytotoxicity of artemisinin-triazine hybrids - Article 3

To be submitted.

To be submitted to: *Bioorganic and Medicinal Chemistry*.

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Theunis T. Cloete[a], Julie A. Clark[b], Michele C. Connelly[b,] Amy Matheny[b], Martina S. Sigal[b], R. Kiplin Guy[b], David D. N'Da[a]*

[a] Department of Pharmaceutical Chemistry, North-West University, Potchefstroom 2520, South Africa

[b] Department of Chemical Biology and Therapeutics, St Jude Children's Research Hospital, 262 Danny Thomas Place, Mailstop 1000, Memphis, TN 38105-3678, United States of America

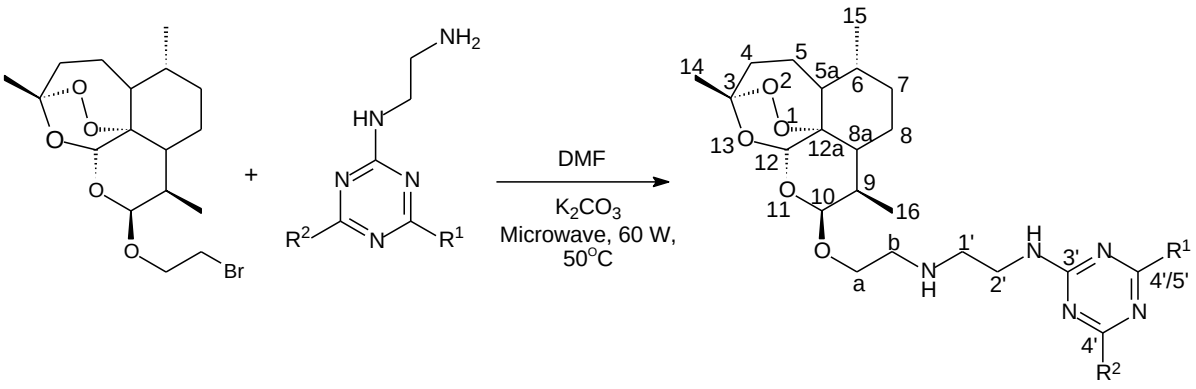
* Corresponding author: David D. N'Da

Tel: + 2718 299 2516; fax: +2718 299 4243

E-mail: david.nda@nwu.ac.za

Graphical abstract

A series of artemisinin-triazine hybrids were synthesised and their antimalarial activity and cytotoxicity were determined *in vitro* against various cell lines.



Synthesis, antimalarial activity and cytotoxicity of artemisinin-triazine hybrids

Theunis T. Cloete[a], Julie A. Clark[b], Michele C. Connelly[b], Amy Matheny[b], Martina S. Sigal[b], R. Kiplin Guy[b], David D. N'Da[a]*

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[b] Department of Chemical Biology and Therapeutics, St Jude Children's Research Hospital, 262 Danny Thomas Place, Mailstop 1000, Memphis, TN 38105-3678, United States of America

* Corresponding author: David D. N'Da
Tel: + 2718 299 2516; fax: +2718 299 4243
E-mail: david.nda@nwu.ac.za

Abstract

In this study seven artemisinin-triazine hybrids were synthesised and their *in vitro* antimalarial activity against both the chloroquine sensitive (3D7) and resistant (K1) strains of *Plasmodium falciparum* was determined as well as their toxicity against human embryonic kidney- (HEK 293), hepatocellular carcinoma- (Hep G2), B-lymphocyte- (Raji) and human fibroblast (BJ) cells. The compounds were prepared by linking the artemisinin and triazine pharmacophores through an amino-ethylamino-ethylether chain using conventional and microwave assisted methods, and their structures were confirmed by NMR and HRMS. The synthesised hybrids were active against both strains. However, compound **17**, featuring *p*-anisidine and 2-(diisopropylamino)ethylamine substituents on the triazine ring was the most active of all. With an EC_{50} value of 1.8 nM against both the 3D7 and K1 strains, compound **17** was found to possess activity comparable to that of artesunate (AS) and artemether (AM) while being significantly more potent than chloroquine (EC_{50}: 13.2 and 1670.9 nM against 3D7 and K1, respectively). The hybrids were found to be non-toxic against HEK 293, Hep G2, Raji and BJ cells. With good activity and low toxicity, compound **17** is a good drug candidate to be further investigated. It will be interesting to ascertain if the same nanomolar activity would be expressed *in vivo,* and whether its mechanism of action is purely dependent on the artemisinin pharmacophore or it the folate biosynthetic pathway is also implicated.

Keywords: Artemisinin, triazine, hybrids, microwave, antimalarial, cytotoxicity

5.1 Introduction

Malaria has been a major parasitic disease of man since antiquity and is still an enormous problem. Despite our increased knowledge of the disease, there are still 6.6 billion people are at risk of malaria with an estimated 655 000 people that die annually.[1] The five species of the *Plasmodium* parasite that have the capability to infect humans are *P. vivax*, *P. ovale*, *P. malariae*, *P. knowlesi* and *P. falciparum* with the latter accounting for 91 % of all reported cases.[1]

Currently used antimalarial drugs fall into one of seven classes, *viz*. the 4-aminoquinolines, the arylaminoalcohols, the 8-aminoquinolines, the antifolates, the hydroxynaphthoquinones, certain antibiotics (e.g. doxycyclin and clindamycin) and the artemisinin class of compounds consisting of artemisinin (ART), dihydroartemisinin (DHA), artemether (AM) and artesunate (AS).[2] Unfortunately, due to the parasite's amazing ability to acquire resistance against most of these drugs, only the artemisinin class of compounds remain as a viable first line treatment option for uncomplicated *P. falciparum* malaria in most of the areas afflicted by the disease.[3]

Although the artemisinin class of compounds' mechanism of action is still under investigation, these compounds have proved to be invaluable in the fight against malaria mainly because of their extremely fast and potent antimalarial action, the lack of cross resistance with other antimalarial drugs and their action against the gametocyte forms of the parasite.[4] Despite the clear advantages of these compounds, they do however suffer from poor water and oil solubility, a short plasma half life and high parasite recrudescence after treatment.[5]

In an effort to enhance the efficiency of the antimalarial drugs and to counter the spread of resistance, the artemisinin class of compounds are now combined with other antimalarials of a distinct different class to form artemisinin-based combination therapies (ACTs). These ACTs entail combining a fast-acting highly effective artemisinin derivative, e.g. AS, with a longer acting partner drug, e.g. mefloquine, to form a combination that is highly effective in killing the parasite delaying the onset of resistance.[6] The World Health Organisation (WHO) now recommends using an ACT as the first line treatment when uncomplicated *P. falciparum* malaria is diagnosed.[7] Unfortunately, even with these precautions in place, resistance against AS has already been observed in patients from Pailin in western Cambodia.[8] With the artemisinin class of compounds being the foundation on which treatment regimes are currently built, the loss of this important drug class would have dire consequences.

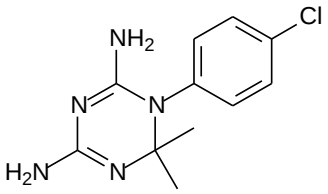
Another approach to counter the development of resistant parasite strains is the synthesis of hybrid drug molecules. A hybrid drug molecule is defined as a chemical

entity with more than one structural domain, each having its own mechanism of action. Aside from the obvious advantage of these types of molecules that is the dual mechanism of action, other advantages may include more predictable pharmacokinetic properties and the ability of one entity to impart favourable qualities onto the other entities, e.g. increased solubility.[9]

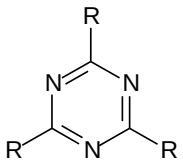
In this study, hybrid molecules consisting of artemisinin and triazine moieties are proposed as possible structures to address the shortcomings of the artemisinin drugs. The 1,3,5-triazine moiety is a common structure found in antifolate drugs (Fig. 1). With various different substituents, the 1,3,5-triazine moiety has been found to have relative good activity against malaria and other bacteria both on its own and whilst attached to other pharmacophores.[10-12,13,14]

The folate biosynthetic pathway in the parasite consists of a process in which six enzymes are involved in the conversion of guanosine triphosphate (GTP) to tetrahydrofolic acid (THF), which is of vital importance in the synthesis of purines, thymidine and some amino acids.[15] The folate antagonists, also known as antifolates, can be divided into two classes, *viz*. type 1 and type 2 antifolates. The type 1 antifolates consist of sulfonamides and sulfones that mimic *p*-aminobenzoic acid (PABA), competing for the active site on dihydropteroate synthase (DHPS) consequently inhibiting the formation of dihydropteroate. The type 2 antifolates on the other hand, consist of pyrimethamine, quinazolines, biguanides and triazine metabolites. These antifolates inhibit dihydrofolate reductase (DHFR) preventing the nicotinamide adenine dinucleotide phosphate (NADPH) dependent reduction of dihydrofolate (DHF) to THF.[16] Not long after their introduction into the marked, worldwide resistance against this class spread rapidly making them obsolete in most areas afflicted by malaria.[17] Combination of both type 1 and type 2 antifolates, e.g. sulfadoxine and pyrimethamine (SP), proved to have a synergistic effect against malaria, though resistance against even these combinations developed quickly preventing them from being used on a great scale against malaria.[18]

The compounds described in this article contain the artemisinin and 1,3,5-triazine pharmacophores and are designed to be active against both the sensitive and resistant strains of *P. falciparum*. We herein report their synthesis, *in vitro* antimalarial activity against various strains of *P. falciparum* and cytotoxicity against four mammalian cell-types.



Cycloguanil



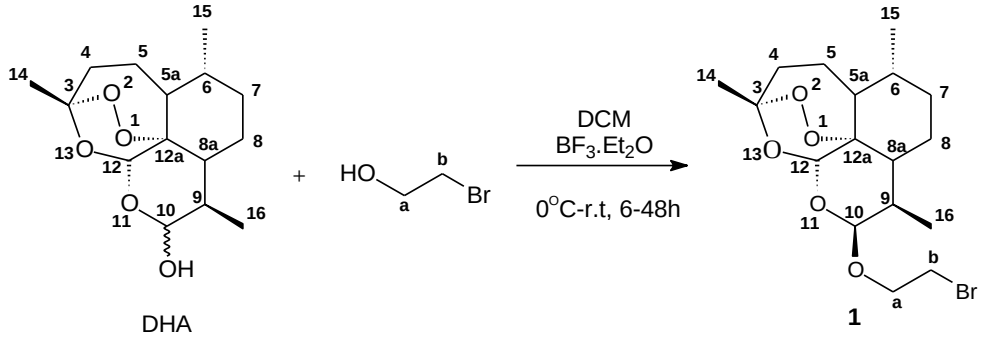
2, 4, 6-trisubstituted-1, 3, 5-triazine moiety

Figure1: Structures of cycloguanil and the 2,4,6-trisubstituted-1,3,5-triazine moiety

5.2 Results

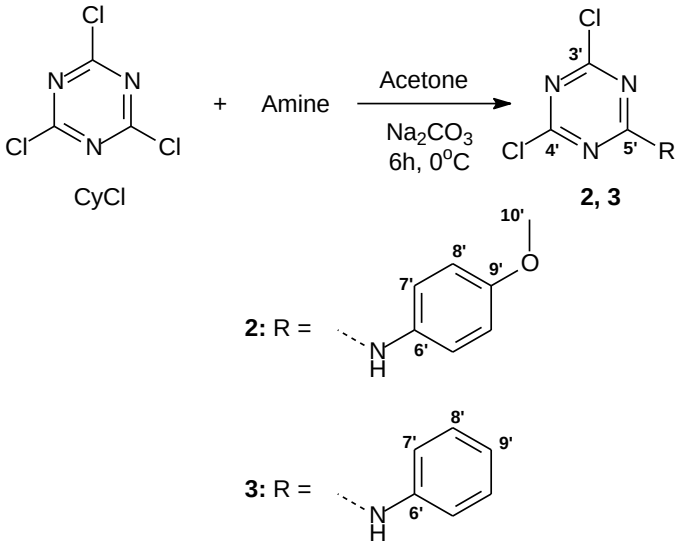
5.2.1 Chemistry

Condensation of dihydroartemisinin with 2-bromoethanol produced the DHA-ethyl bromide derivative (**1**) in good yield (Scheme 1).[19,20] This intermediate and the final hybrids were all in the 10β form, as is confirmed by the coupling constant *J* = 3.4 Hz between H-10 and H-9.[21] The stereochemistry was previously determined in our group by X-ray analysis.[22]

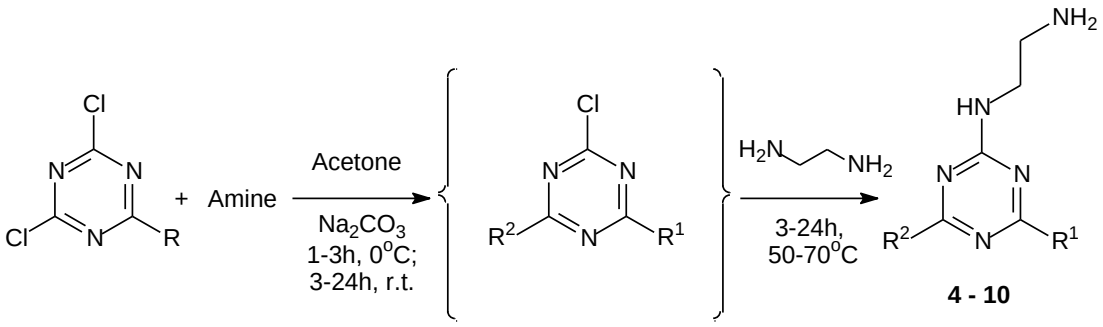


Scheme 1. Etheration of DHA yielding compound **1**.

CyCl (cyanuric chloride) was then reacted with the relevant amine to produce the monosubstituted compounds **2** and **3** with yields of 68 and 86 %, respectively (Scheme 2). Subsequently, one of these two monosubstituted compounds or CyCl was reacted with different amines, generating the disubstituted compounds that were used in the following reaction to form the primary amino-functionalised trisubstituted triazine intermediates (**4** – **10**) with yields ranging from 49 to 100 % (Scheme 3).



Scheme 2. Monosubstitution of CyCl.



Scheme 3. Amination of CyCl, **2** and **3** (R^1 & R^2: **4 ≡ 11**; **5 ≡ 12**; **6 ≡ 13**; **7 ≡ 14**; **8 ≡ 15**; **9 ≡ 16**; **10 ≡ 17**).

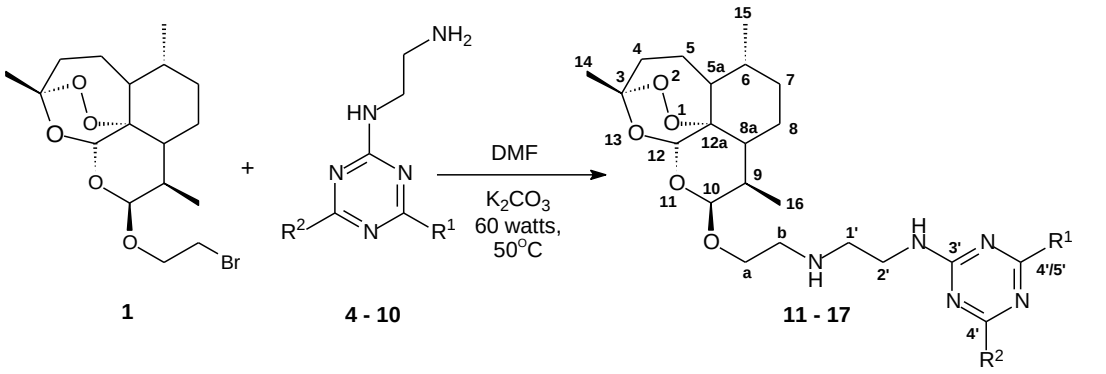
Finally, compounds **4** – **10** were reacted with compound **1** using the described method with microwave radiation rendering hybrids **11** - **17** with yields in the 11 – 28 % range (Scheme 4).

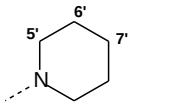
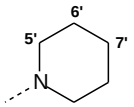
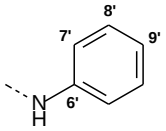
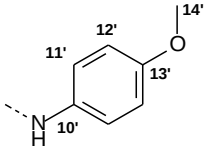
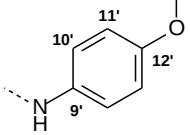
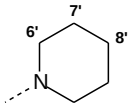
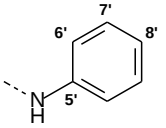
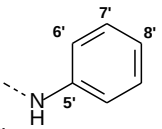
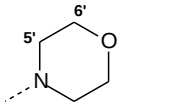
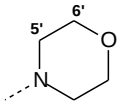
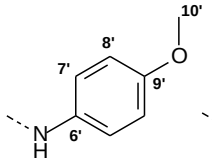
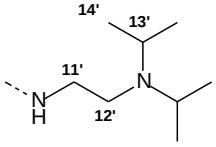
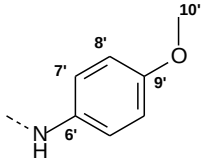
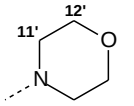
5.2.2 Biological activity

The antimalarial activity and cytotoxicity of the synthesised compounds are given in Table 1. EC_{50} values 5-fold higher or lower than that of the standard were considered as significantly different while those differing by 10-fold were considered as meaningful. All hybrids were active against both strains.

Against the CQS 3D7 strain compound **17** had activity significantly better than chloroquine (CQ) while compounds **11** – **16** had similar activity. Compounds **13** and

17 had activity comparable to that of AS and AM whilst compound **14** had activity comparable to AS but was significantly weaker than AM. Compounds **11** and **16** were both significantly weaker than AS and meaningfully weaker than AM. Compounds **12** and **15** had the lowest activity of the synthesised compounds being meaningfully weaker than AS and AM.



Compound	R^1	R^2	Compound	R^1	R^2
11			**15**		
12			**16**		
13			**17**		
14					

Scheme 4. Synthesis of artemisinin-triazine hybrids **11 – 17**.

Against the CQR K1 strain all compounds had activity significantly better than that of CQ. Compounds **13**, **14** and **17** had activity comparable to both AS and AM whilst compound **16** had activity significantly lower than both these drugs. Compounds **11** and **12** both had activity significantly lower than that of AS and meaningfully lower than the more active AM. Compound **15** once again had activity meaningfully lower than that of both AS and AM.

Table 1. *In vitro* antimalarial activity of compounds **11 – 17** against the 3D7 and K1 strains of *Plasmodium falciparum*, and their cytotoxicity against HEK 293, Hep G2, B-lymphocyte (Raji) and human fibroblast (BJ) cell lines.
Cells were incubated with compounds at various concentrations for 72 h; antimalarial activity and cytotoxicity were determined using SYBR Green I-based fluorescence and CellTiter-Glo luminescent cell viability assays, respectively.

			Activity EC_{50} (nM)[a]			**Cytotoxicity EC_{50} (μM)[b]**				**Selectivity Index**			
Cpd	**logP[c]**	**pK_a[c]**	**3D7**	**K1**	**RI[d]**	**HEK 293**	**Hep G2**	**BJ**	**Raji**	**SI_1[e].10^3**	**SI_2[f].10^3**	**SI_3[g].10^3**	**SI_4[h].10^3**
11	6.7	6.9 8.6	25.7	24.7	1	>28.9	6.4	>29	>29	>1.1	0.3	>1.1	>1.1
12	3.8	6.4 8.6	32.3	26.2	0.8	>29.9	2.9	>29.9	>29.9	>0.	88.10	>0.9	>0.9
13	3.6	6.4 8.6	3.8	3	0.8	>33.8	>33.8	>33.8	>33.8	>8.9	>8.9	>8.9	>8.9
14	2.2	6.2 8.6	8.4	5.5	0.7	>32.5	9.5	>3.23	>32.5	>3.9	1.1	>3.9	>3.9
15	4.8	4.9 8.5	36.7	30.8	0.8	>33.6	6.3	26.0	>33.6	>0.9	0.2	0.7	>0.9
16	4.8	4.9 8.5	24.9	17.4	0.7	7.9	4.7	12.9	>26.6	0.3	0.2	0.5	>1.1
17	4.9	8.6 10.2	1.8	1.8	1	10.1	4.8	13.6	>26.3	5.6	2.7	7.5	>14.6
AS	3.03	4.3	3.2	2.8	0.9	>26.0	>26.0	>26.0	>26.0	>8.1	>8.1	>8.1	>8.1
AM	2.97	NA	1.8	2.3	1.3	>26.0	>26.0	>26.0	>26.0	>14.5	>14.5	>14.5	>14.5
CQ†	4.69	7.5 10.4	13.2	1 670.9	126.6	>20.2	>20.2	>23.8	0.1	>1.5	>1.5	>1.8	0.0
STP	nd	nd	nd			2.3	2.0	nd	nd				

[a] Effective concentration of compound inducing 50% reduction in parasitic cells count; [b] Effective concentration of compound selectively inducing 50% reduction in parasitic cells count in the presence of mammalian cells; [c] Calculated values[28]; [d] Resistance index (RI) = EC_{50} K1 / EC_{50} 3D7; [e] Selectivity index (SI_1) = EC_{50} HEK 293/EC_{50} 3D7; [f] Selectivity index (SI_2) = EC_{50} Hep G2/EC_{50} 3D7; [g] Selectivity index (SI_3) = EC_{50} BJ /EC_{50} 3D7; [h] Selectivity index (SI_4) = EC_{50} Raji G2/EC_{50} 3D7; †CQ was tested as diphosphate salt; Staurosporine (STP) was used as the reference drug in the cytotoxicity study; nd = not determined

The synthesised compounds do not possess any meaningful cytotoxicity with EC_{50} values well above that of the reference drug staurosporine on both human embryonic kidney cells (HEK 293), hepatocellular carcinoma cells (Hep G2), B-lymphocyte cells (Raji) and human fibroblast cells (BJ) (Table 1).

5.3 Discussion

5.3.1 Chemistry

The artemisinin-triazine hybrids were synthesised using microwave radiation and their structures were confirmed using HRMS and NMR techniques. The microwave reaction was carried out using a 250 ml open vessel round bottom flasks connected to a liebig cooler, with a ramp and hold time of 1 and 4 minutes respectively. The maximum temperature and watts allowed was set at 50 °C and 60 watts and carried out by increasing the watts until either the temperature or the 4 minutes hold time was reached. During each cycle cooled air was pumped into the reactor, keeping the temperature as low as possible. After each cycle the reaction vessel was cooled to -8 °C for the next burst. These burst were repeated 8 – 13 times, until TLC showed that the reaction was complete.

The peroxide bridge is reported to be susceptible to facile hydrolysis under basic and acidic conditions and can also be expected to be sensitive to heat.[23] ^{13}C NMR spectrometry and high resolution MS, however, confirmed that the peroxide bridge was preserved during microwave radiation, with no deviations from the expected values. This correlated well with previous findings that microwave radiation decreased the overall reaction time, but had no noticeable influence on the yield of the reactions.[20]

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

The ^{1}H NMR signals of the ethylenediamine (EDA) linker can be seen at δ 3.10 and 3.60 for H-1' and H-2', respectively. The H-1' signal is usually in close proximity to the H-b signal at δ 3.25, together integrating for four protons. The COSY is paramount to the correct allocation of these two signals as H-b couples with H-aα and H-aβ, whilst H-1' couples with H-2'. The ^{13}C NMR signals of C-1' and C-b can be seen at δ 47. The HSQC spectrum assists in confirming the presence of these two signals. The piperidine moiety of compounds **11** and **12** has distinctive ^{1}H NMR signals at δ 3.72 and 1.71 arising from the alicyclic protons while the alicyclic carbon atoms display signals at δ 44.29 and 26.20. The morpholine moiety of compounds **13** and **14** has ^{1}H NMR signals at δ 3.71 – 3.41 correlating to the ^{13}C NMR signals at δ 66.04 and 43.18 in the HSQC spectrum. The *p*-anisidine part of compounds **12**, **14**, **15** and **17** shows the characteristic methoxy signal at δ 3.72 and the two aromatic doublets at

δ 7.45 and 6.75 in the ^{1}H NMR spectrum. The methoxy carbon signal can be found at δ 55.45 in the ^{13}C NMR spectrum whilst the aromatic carbon atoms resonate at δ 153.61, 129.89, 123.16 and 113.70. The aniline moiety of compounds **15** and **16** displays signals in the aromatic region at δ 7.48, 7.20 and 7.01 in the ^{1}H NMR spectrum and at δ 120.05, 121.59, 128.53 and 140.11 in ^{13}C NMR. The four methyl groups of the 2-(diisopropylamino)ethylamine moiety in compound **17** give a characteristic ^{1}H NMR signal at δ 1.06 ppm corresponding to the ^{13}C NMR signal at δ 20.24 while the signals resulting from the two methine groups give a ^{1}H NMR signal at δ 2.68 and a ^{13}C NMR signal at δ 44.19.

5.3.2 Biological activity

Only the artemisinin-triazine hybrids (**11** – **17**) were evaluated against malaria. Artemisinin and the triazine compounds are known to be active against malaria, either on their own or as the pharmacophore in other drug compounds.[10-14,24,25] *In vivo*, most artemisinin derivatives are metabolised into DHA within a few minutes.[25-27] AM and arteether, both ether derivatives of artemisinin, stay intact long enough to exercise their own antimalarial effect before being metabolised into DHA, with all three drugs showing comparable potency.[25] The synthesised hybrids, also being ether derivatives of artemisinin, will thus likely also exert their antimalarial activity on their own.

The resistance index (RI) of compounds **12** – **16** is smaller than 1, an indication that these compounds are slightly more active against the CQR strain than against the CQS strain, which is an interesting observation. Also noteworthy is that the order of the EC_{50} values of the hybrids against the sensitive strain is identical to that against the resistant strain (**17** < **13** < **14** < **16** < **11** < **12** < **15**).

Another interesting observation is that compound **17**, displaying the lowest EC_{50} value of the hybrids, has a tertiary amine with a calculated pK_a value of 10.2,[28] which is comparable to the calculated pK_a value of 10.4 of CQ. One of the suggested mechanisms of action of CQ is ion trapping, which relies on the action of amino groups as weak basis. In the normal *in vivo* environment at pH 7.4, amines tend to be neutral and unprotonated making it easy for them to cross lipophillic membranes. When these unprotonated amines reach the acidic food vacuole of the malaria parasite at a pH of 4.5 - 5.5, they become protonated making them less membrane permeable. The net effect of this mechanism is the increased accumulation of these amines in the digestive vacuole, giving rise to an increase in activity.[29,30] This observation might thus imply that, as with CQ, ion trapping plays a role in this compound's activity.

Compounds **11** and **13** are homologous differing only in respect to the nitrogen atom of the heterocyclic substituents on the triazine moiety, causing a 7-fold increase in activity from **11** to

13. This same is seen in compounds **12** and **14** where a 4-fold increase in activity is observed from **12** to **14**. The pK_a values within these pairs of compounds are roughly the same, but the calculated log P value of **11** is higher than that of **13** (6.7 *vs.* 3.6) and likewise the calculated log P value of **12** is higher than that of **14** (3.8 vs. 2.2).[28] Thus, the compound containing the oxygen atom has lower EC_{50} and log P values. This might be explained by the findings of Lipinski stating that a log P value in the 0-5 range are acceptable while a value in the 0-3 range is ideal for absorption through biological membranes.[31,32]

Against both strains, none of the compounds had activity higher than that of AS or AM. Therefore, while the addition of the triazine moieties to artemisinin had various positive effects, 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.[33]

The cytotoxicity of compounds **11 – 17**, AS and AM were determined against HEK 293, Hep G2, Raji and BJ cells using staurosporine (STP) as reference drug. Most of the hybrids were less toxic than both AS and AM, and all were less toxic than the reference drug (Table 1). The target hybrids thus exhibit a high degree of selectivity towards the malaria parasite.

5.4 Conclusion

In this study a series of seven hybrids were successfully synthesised by linking the artemisinin and triazine pharmacophores through an amino-ethylamino-ethylether chain. Their structures were confirmed by HRMS and NMR techniques.

The synthesised hybrids had potencies comparable to that of CQ against the 3D7 strain except compound **17**, which was found to be significantly more potent. In comparison to artesunate and artemether against the 3D7 strain, hybrids **13** and **17** had comparable activity whilst **14** was comparable to artesunate while being slightly less potent than AM. Compounds **11**, **12**, **15** and **16**, on the other hand, were either significantly or meaningfully less active than both AS and AM. Against the K1 strain, however, all the hybrids were significantly more active than CQ, with compounds **13**, **14** and **17** once again displaying potency comparable to that of AS and AM. Against the K1 strain hybrids **11**, **12**, **15** and **16** once again were either significantly or meaningfully less potent than AS and AM.

Compound **17**, the most active hybrid against both strains, resembles CQ in having an amino group with a pK_a value of 10.4. This feature may, through ion trapping, be responsible for the additional activity observed for this compound.

The hybrids were found to be non-toxic to all HEK 293, Hep G2, Raji and BJ mammalian cells.

In summary, this study yielded two compounds *viz.* **13** and **17**, which did not show noticeable increase but rather comparable potency to both artesunate and artemether. However, the conclusions drawn on the basis of their structural and physical chemical properties may qualify them as excellent leads to be further investigated. It would be interesting to establish whether their excellent *in vitro* nanomolar activity will also be apparent *in vivo*, and whether their mechanism of action is purely dependent on the artemisinin pharmacophore or if the folate biosynthetic pathway is also implicated.

Furthermore, these hybrids stand as good candidates to undergo pharmacokinetic studies, to establish whether they possess half-lives longer than those of the current clinically available artemisinin derivatives that are known to have short biological half-lives.

5.5 Materials and methods

5.5.1 Materials

Dihydroartemisinin (mixture of 10α and 10β epimers) was purchased from Changzhou Kaixuan Chemical Co (Chunjiang, China). Boron trifluoride diethyl etherate ($BF_3.Et_2O$), 2-bromoethanol, N,N-dimethylformamide (DMF), morpholine, piperidine, aniline, cyanuric chloride (CyCl), tetrahydrofuran (THF), ethylenediamine (EDA) and 2-(diisopropylamino)ethylamine were purchased from Sigma-Aldrich (Johannesburg, South Africa). *p*-Anisidine was purchased from Merck Schuchardt (Hohenbrunn, Germany). Oxalic acid was purchased from BDH laboratory reagents (Yorkshire, England). Sodium carbonate and potassium carbonate was purchased from Unilab (Krugersdorp, South Africa). All the chemicals and reagents were of analytical grade and were used without further purification.

5.5.2 General chemical analytical procedures

The ^{1}H 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-*d6*) or deuterated chloroform ($CDCl_3$-*d*). 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), t (triplet), td (triplet of doublets), q (quartet), m (multiplet).

Mass spectra (MS) were recorded in positive mode by one of two mass spectrometers. The first MS was a Thermo Electron LXQ™ ion trap mass spectrometer that 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 sec, with a capillary voltage of 7 V while the corona discharge

was 10 µA. The second MS was a Bruker MicroTOF Q II mass spectrometer that had an APCI or an ESI source set at 300°C or 180°C respectively, with Bruker Compass DataAnalysis 4.0 software. A full scan from 50 - 1500 m/z was obtained with a capillary voltage of 4500 V, an end plate offset voltage of -500 V and a collision cell RF voltage of 100 Vpp.

The melting points (Mp) were determined in duplicate on a BÜCHI melting point B-545 instrument and are reported in degree Celsius (°C) uncorrected. Thin layer chromatography was performed using silica gel plates ($60F_{254}$). Column chromatography was carried out on silica gel (230-240 mesh, G60 Merck) and silica gel 60 (70-230 mesh ASTM, Fluka).

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.

5.5.3 Biological study

5.5.3.1 Determination of antimalarial effective concentration

The synthesised compounds were screened against the chloroquine-sensitive (CQS, 3D7) and chloroquine-resistant (CQR, K1) strains of *P. falciparum*. Continuous *in vitro* cultures of asexual erythrocyte stages of *P. falciparum* were maintained using a modified method of Trager and Jensen.[34] Asynchronous parasites were grown in the presence of fresh group O-positive erythrocytes (Lifeblood, Memphis, TN) in Petri dishes at a hematocrit of 4-6% in RPMI-based medium consisting of RPMI 1640 supplemented with 0.5% AlbuMAX II, 25 mM HEPES, 25 mM $NaHCO_3$ (pH 7.3), 100 µg/mL hypoxanthine, and 5 µg/mL gentamicin. Cultures were incubated at 37 °C in a gas mixture of 90% N_2, 5% O_2, and 5% CO_2. For EC_{50} determinations, 20 µL of RPMI 1640 with 5 µg/mL gentamicin were dispensed per well in an assay plate (384-well microtiter plate, clear-bottom, tissue-treated). Next, 40 nL of each compound, previously serially diluted in a separate 384-well white polypropylene plate, was dispensed in the assay plate, and then 20 µL of a synchronized culture suspension (1% rings, 10% hematocrit) was added per well to make a final hematocrit and parasitemia of 5% and 1%, respectively. Assay plates were incubated for 72 h, and the parasitemia was determined by a method previously reported.[35] Briefly, 10 µL of 10X Sybr Green I, 0.5% v/v Triton, and 0.5 mg/mL saponin solution in RPMI were added per well. Assay plates were shaken for 30 s,

incubated in the dark for 4 h, and then read with the Envision spectrofluorometer at Ex/Em 485 nm/535 nm. EC_{50} values were calculated using proprietary software developed in house (RISE) in the Pipeline Pilot environment that pools data from all replicates of the experiment and fits a consensus model.

5.5.3.2 *In vitro* cytotoxicity against HEK 293, HEP G2, Raji and BJ cells

Human embryonic kidney cells (HEK 293), hepatocellular carcinoma cells (Hep G2), B-lymphocyte cells (Raji) and human fibroblast cells (BJ) cell lines were purchased from the American Type Culture Collection (ATCC, Manassas, VA) and were cultured according to recommendations. Cell culture media were purchased from ATCC. Cells were routinely tested for mycoplasma contamination using the MycoAlert Mycoplasma Detection Kit (Lonza). Exponentially growing cells were plated in Corning 384 well white custom assay plates, and incubated overnight at 37 ºC in a humidified, 5% CO_2 incubator. DMSO inhibitor stock solutions were added the following day to a top final concentration of 25 µM, 0.25% DMSO and then diluted 1/3 for a total of ten testing concentrations. Cytotoxicity was determined following a 72 h incubation using Promega Cell Titer Glo Reagent according to the manufacturer's recommendation. Luminescence was measured on an Envision plate reader (Perkin Elmer). EC_{50} values were calculated using proprietary software developed in house (RISE) in the Pipeline Pilot environment that pools data from all replicates of the experiment and fits a consensus model.

5.5.3.3 Dose-response curve fitting

Dose-response curves were calculated from percent activity values and log 10-transformed concentrations, the proprietary robust interpretation of screening experiments (RISE) application written in Pipeline Pilot (Accelrys, v. 8.5) and the R program.[36] Briefly, non-linear regression was performed using the R *drc* package with the four-parameter log-logistic function (LL2.4).[37] The data for all replicates for each compound was fit three separate times by varying the parameters that were fixed during regression: (1) all parameters free, (2) high response fixed to 100, (3) low response fixed to 0. The best fit from these three nested models was selected using the *anova.drc* function. 95% confidence intervals were produced based upon this fit.

Dose-response curves were assigned a quality score according to the following heuristic. Compounds which failed to fit to any curve, or with curves having efficacy <25% or >150% or hill slope <0.5 or >25 were designated class 'D1'. Compounds passing this first criteria with curves having efficacy <50%, calculated EC_{50} > the highest concentration tested, lower and

upper EC50 confidence limits > 10-fold EC_{50}, or slope at the highest concentration tested >75% (non-saturating) were designated class 'C1'. Compounds passing previous criteria with curves having lower and upper EC_{50} confidence limits > 5-fold EC_{50} or slope at the highest concentration tested >25% (not completely saturating) were designated class 'B1'. All remaining curves were designated 'A1', which is indicative of ideal, well-behaved sigmoidal response. Curves that were inverted (activity decreased as concentration increased) were prefixed with the letter 'N', such as 'NA1'. In tabulating data, EC_{50} values are only called for A1 and B1 class curves with a single value and error being provided for A1 curves and a range provided for B1 curves. C1 and D1 curves were assigned an arbitrary value of either greater than the highest concentration tested or lower than the lowest concentration tested, when appropriate.

5.5.4 Synthetic procedure

5.5.4.1 2-bromo-(10β-dihydroartemisinoxy) ethane, 1

The 1-bromo-2-(10β-dihydroartemisinoxy)ethane (**1**) analogue of DHA was synthesised by a method similar to that of Li and co-workers (Scheme 1) and has been characterised before.[19]

5.5.4.2 Monoamine substituted triazines 2-3

To a solution of CyCl dissolved in acetone at 0°C, was firstly added Na_2CO_3 and then the relevant amine divided into four portions (Scheme 2). The reaction mixture was allowed to stir for 6 hours at 0°C until completion, after which the reaction was stopped by removing all the solvent *in vacuo*. The resulting solid was washed with ice water (3 × 50 ml), filtered and then purified by column chromatography eluting with various ratios of ethyl acetate (EtOAc), petroleum ether (PE) and dichloromethane (DCM). In some instances the product was then further purified *via* crystallisation from DCM.

5.5.4.2.1 4,6-dichloro-N-(4-methoxyphenyl)-1,3,5-triazin-2-amine, 2

A solution of CyCl (43.4 mmol, 8 g), Na_2CO_3 (86.8 mmol, 9.2 g, 2 eq.) and *p*-anisidine (43.4 mmol, 5.3 g, 1 eq.) in acetone (80 ml) was allowed to stir for 6 hours. Purification by silica gel column chromatography eluting with EtOAc/PE (1:4) then recrystallisation from DCM afforded 8.0 g (68%) of **2** as a brown powder, m.p. 170.9°C; $C_{10}H_8Cl_2N_4O$. ^{1}H NMR (600 MHz, DMSO) δ 7.55 – 7.28 (m, 2H, H-7'), 7.02 – 6.86 (m, 2H, H-8'), 3.74 (s, 3H, H-10'). ^{13}C NMR (151 MHz, DMSO) δ 169.62 (C-3'), 168.59 (C-4'), 163.56 (C-5'), 156.68 (C-9'), 129.51 (C-6'), 123.31 (C-

7'), 114.08 (C-8'), 55.31 (C-10'). COSY & HSQC (See annexure B). HRAPCI-MS *m/z* $[M+H]^+$: 271.0148 (Calcd. for $C_{10}H_9Cl_2N_4O$: 271.0175).

5.5.4.2.2 4,6-dichloro-N-phenyl-1,3,5-triazin-2-amine, 3

A solution of CyCl (21.7 mmol, 4 g), Na_2CO_3 (43.4 mmol, 12.4 g, 2 eq.) and aniline (21.7 mmol, 1.97 ml, 1 eq.) in acetone (50 ml) was allowed to stir for 6 hours. Purification by silica gel column chromatography eluting with DCM afforded 4.5 g (86%) of **3** as a white powder, m.p. 199.5°C; $C_9H_6Cl_2N_4$. ^{1}H NMR (600 MHz, DMSO) δ 7.56 (dd, *J* = 25.2, 8.1 Hz, 2H, H-7'), 7.37 (dt, *J* = 15.6, 7.9 Hz, 2H, H-8'), 7.16 (dt, *J* = 12.2, 7.4 Hz, 1H, H-9'). ^{13}C NMR (151 MHz, DMSO) δ 169.59 (3'), 168.79 (C-4'), 163.84 (C-5'), 136.79 (C-6'), 129.04 (C-8'), 124.86 (C-9'), 121.77 (C-7'). COSY & HSQC (See annexure B). HRAPCI-MS *m/z* $[M+H]^+$: 241.0028 (Calcd. for $C_9H_7Cl_2N_4$: 241.0070).

5.5.4.3 Primary amino-functionalized triazines 4-10

The synthesis occurred in two steps. First, to a solution of either CyCl or monosubstituted CyCl dissolved in acetone at 0°C was firstly added Na_2CO_3 and then the relevant amine divided into four portions (Scheme 3). The reaction mixture was allowed to stir for 1 - 3 hours at 0°C and then at 3 - 24 hours at room temperature after which the reaction was stopped by removing the solvent *in vacuo*. The resulting residue was dissolved in either EtOAc or DCM and washed with water, and then purified by column chromatography eluting with various ratios of methanol (MeOH), EtOAc, DCM, PE and NH_4OH. The resulting product was then used without further purification.

In the second step, the above synthesised disubstituted triazine intermediate was dissolved in EDA and heated with continuous stirring at 50 - 70°C for 3 - 24 hours. Afterwards, the reaction was quenched with ice cold water and the crude product was extracted with EtOAc (3 x 100 ml) and purified by column chromatography eluting with various ratios of MeOH, DCM, and ammonia (NH_4OH).

5.5.4.3.1 N-(2-aminoethyl)-4,6-bis(piperidin-1-yl)-1,3,5-triazin-2-amine, 4

A solution of CyCl (21.7 mmol, 4 g), Na_2CO_3 (43.4 mmol, 4.6 g, 2 eq.) and piperidine (43.4 mmol, 4.3 ml, 2 eq.) in acetone (60 ml) was allowed to stir for 2 hours in an ice bath, then 24 hours at room temperature followed by extraction with DCM. Purification by silica gel column chromatography eluting with DCM afforded 3.11 g of the intermediate as a white powder.

The reaction of the intermediate (7.1 mmol, 2 g) with EDA (224.1 mmol, 15 ml, 31.5 eq.) at 60°C was stirred for 24 hours. After completion extraction with EtOAc and then successive

purification with silica gel column chromatography eluting with MeOH:DCM:NH_4OH (1:13.5:0.5) then MeOH:NH_4OH (19:1) afforded 1.8 g (83%) of **4** as a white powder, m.p. 123.2°C; $C_{15}H_{27}N_7$: ^{1}H NMR (600 MHz, $CDCl_3$) δ 3.62 (s, 8H, H-5'), 3.43 – 3.34 (m, 2H, H-2'), 2.83 (dd, *J* = 16.6, 10.9 Hz, 2H, H-1'), 1.58 – 1.42 (m, 14H, H-7', H-6'). ^{13}C NMR (151 MHz, $CDCl_3$) δ 166.58 (C-3'), 164.89 (C-4'), 44.00 (C-5'), 42.99 (C-2'), 41.95 (C-1'), 25.77 (C-6'), 24.83 (C-7'). COSY & HSQC (See annexure B). HRAPCI-MS *m/z* $[M+H]^+$: 306.2390 (Calcd. for $C_{15}H_{28}N_7$: 306.2430).

5.5.4.3.2 2N-(2-aminoethyl)-4N-(4-methoxyphenyl)-6-(piperidin-1-yl)-1,3,5-triazine-2,4-diamine, 5

A solution of **2** (14.8 mmol, 4 g), Na_2CO_3 (29.6 mmol, 3.1 g, 2 eq.) and piperidine (14.8 mmol, 1.5 ml, 1 eq.) in acetone (40 ml) was allowed to stir for 1 hour in an ice bath, then 16 hours at room temperature followed by extraction with EtOAc. Purification by silica gel column chromatography eluting with EtOAc:PE (1:4) afforded 5.6 g of the intermediate as an orange oil.

The reaction of the intermediate (17.5 mmol, 5.6 g) with EDA (373.5 mmol, 25 ml, 21.3 eq.) at 65°C was stirred for 22 hours. After completion extraction with EtOAc and purification with silica gel column chromatography eluting with MeOH:DCM:NH_4OH (1:14:0.5) afforded 3.5 g (58%) of **5** as a white powder, m.p. 132.8°C; $C_{17}H_{25}N_7O$: ^{1}H NMR (600 MHz, $CDCl_3$) δ 7.42 (d, *J* = 7.5 Hz, 2H, H-10'), 6.81 (d, *J* = 8.9 Hz, 2H, H-11'), 3.90 – 3.54 (m, 10H, H-6', H-13'), 3.42 (dd, *J* = 11.6, 5.8 Hz, 2H, H-2'), 2.85 (t, *J* = 5.8 Hz, 2H, H-1'), 1.67 – 1.48 (m, 9H,H-7', H-8'). ^{13}C NMR (151 MHz, $CDCl_3$) δ 139.00 (C-3'), 164.92 (C-4'), 164.27 (C-5'), 155.13 (C-12'), 132.52 (C-9'), 121.80 (C-10'), 113.58 (C-11'), 55.47 (C-13'), 44.15 (C-6'), 43.35 (C-2'), 41.83 (C-1'), 25.78 (C-7'), 24.82 (C-8'). COSY & HSQC (See annexure B). HRAPCI-MS *m/z* $[M+H]^+$: 344.2175 (Calcd. for $C_{17}H_{26}N_7O$: 344.2221).

5.5.4.3.3 N-(2-aminoethyl)-4,6-bis(morpholin-4-yl)-1,3,5-triazin-2-amine, 6

A solution of CyCl (21.7 mmol, 4 g), Na_2CO_3 (43.4 mmol, 4.5 g, 2 eq.) and morpholine (43.4 mmol, 3.8 ml, 2 eq) in acetone (50 ml) was allowed to stir for 3 hours in an ice bath, then 22 hours at room temperature followed by extraction with DCM. Successive purification with silica gel column chromatography eluting with EtOAc:DCM (1:1) then DCM afforded 4.2 g of the intermediate as a white powder.

The reaction of the intermediate (7.0 mmol, 2 g) with EDA (373.5 mmol, 25 ml, 53.4 eq.) at 65°C was stirred for 24 hours. After completion extraction with EtOAc and then successive purification with silica gel column chromatography eluting with MeOH:DCM:NH_4OH (1:4:0.5)

then MeOH:DCM:NH_4OH (1:9:0.5) afforded 1.0 g (49%) of **6** as a white powder, m.p. 133.2°C; $C_{13}H_{23}N_7O_2$: ^{1}H NMR (600 MHz, $CDCl_3$) δ 3.85 – 3.55 (m, 17H, H-5', H-6'), 3.39 (q, *J* = 6.0 Hz, 2H, H-2'), 2.83 (t, *J* = 5.9 Hz, 2H, H-1'). ^{13}C NMR (151 MHz, $CDCl_3$) δ 166.56 (C-3'), 165.23 (C-4'), 66.59 (C-6'), 43.50 (C-2', C-5'), 41.79 (C-1'). COSY & HSQC (See annexure B). HRAPCI-MS *m/z* $[M+H]^+$: 310.1972 (Calcd. for $C_{13}H_{24}N_7O_2$: 310.2013).

5.5.4.3.4 2-N-(2-aminoethyl)-4-N-(4-methoxyphenyl)-6-(morpholin-4-yl)-1,3,5-triazine-2,4-diamine, 7

A solution of **2** (14.8 mmol, 4 g), Na_2CO_3 (29.5 mmol, 8.5 g, 2 eq.) and morpholine (14.8 mmol, 1.3 ml, 1 eq.) in acetone (40 ml) was allowed to stir for 1 hour in an ice bath, and then 3 hours at room temperature followed by extraction with EtOAc. Purification by silica gel column chromatography eluting with EtOAc:PE (1:1) afforded 4.7 g of the intermediate as an orange powder.

The reaction of the intermediate (6.2 mmol, 2 g) with EDA (373.5 mmol, 25 ml,60.2 eq.) at 58°C was stirred for 6 hours. After completion extraction with EtOAc and then purification with silica gel column chromatography eluting with MeOH:DCM:NH_4OH (1:9:0.5) afforded 1.8 g (85%) of **7** as a white powder, m.p. 154.6°C; $C_{16}H_{23}N_7O_2$: ^{1}H NMR (600 MHz, DMSO) δ 7.59 (s, 2H, H-7'), 6.81 (t, *J* = 9.6 Hz, 2H, H-8'), 3.79 – 3.55 (m, 12H, H-10', H-11', H-12'), 3.49 – 3.19 (m, 2H, H-2'), 2.75 – 2.56 (m, 2H, H-1'). ^{13}C NMR (151 MHz, DMSO) δ 166.79 (C-3'), 165.01 (C-4'), 163.67 (C-5'), 153.79 (C-9'), 133.66 (C-6'), 121.17 (C-7'), 113.52 (C-8'), 66.18 (C-12'), 55.02 (C-10'), 43.27 (C-2', C-11'), 41.16 (C-1'). COSY & HSQC (See annexure B). HRAPCI-MS *m/z* $[M+H]^+$: 346.1965 (Calcd. for $C_{16}H_{24}N_7O_2$: 346.2013).

5.5.4.3.5 2-N-(2-aminoethyl)-4-N-(4-methoxyphenyl)-6-N-phenyl-1,3,5-triazine-2,4,6-triamine, 8

A solution of **3** (8.3 mmol, 2 g), Na_2CO_3 (16.6 mmol, 4.7 g, 2 eq.) and *p*-anisidine (8.3 mmol, 1.02 g, 1 eq.) in acetone (40 ml) was allowed to stir for 1 hour in an ice bath, and then 4 hours at room temperature followed by extraction with EtOAc. Purification by silica gel column chromatography eluting with EtOAc:PE (1:4) afforded 2.3 g of the intermediate as an off white powder.

The reaction of the intermediate (6.1 mmol, 2 g) with EDA (448.3 mmol, 30 ml, 73.5 eq.) at 70°C was stirred for 4 hours. After completion extraction with EtOAc and then purification with silica gel column chromatography eluting with MeOH:DCM:NH_4OH (1:4:0.5) afforded 1.8 g (84%) of **8** as a purple powder, m.p. 134.5°C; $C_{18}H_{21}N_7O$: ^{1}H NMR (600 MHz, DMSO) δ 7.69 (d, *J* = 65.3 Hz, 4H, H-7', H-11'), 7.20 (s, 2H, H-8'), 6.85 (d, *J* = 50.0 Hz, 3H, H-9', H-12'), 3.70

(d, *J* = 15.8 Hz, 3H, H-14'), 3.47 (d, *J* = 91.5 Hz, H-2', H_2O), 3.06 (s, 2H, H-1'). ^{13}C NMR (151 MHz, DMSO) δ 165.88 (C-3'), 163.90 (C-4', C-5'), 154.54 (C-13'), 140.22 (C-6'), 133.07 (C-10'), 128.30 (C-8'), 121.92 (C-9'), 121.20 (C-11'), 119.83 (C-7'), 113.41 (C-12'), 55.05 (C-14'), 40.32 (C-2'), 38.98 (C-1'). COSY & HSQC (See annexure B). HRAPCI-MS *m/z* $[M+H]^+$: 352.1864 (Calcd. for $C_{18}H_{22}N_7O$: 352.1908).

5.5.4.3.6 2-N-(2-aminoethyl)-4-N,6-N-diphenyl-1,3,5-triazine-2,4,6-triamine, 9

A solution of CyCl (27.1 mmol, 5 g), Na_2CO_3 (54.2 mmol, 15.5 g, 2 eq.) and aniline (54.2 mmol, 4.9 ml, 2 eq.) in acetone (80 ml) was allowed to stir for 2 hours in an ice bath, and then 5 hours at room temperature followed by extraction with EtOAc. Purification by silica gel column chromatography eluting with DCM afforded 5.9 g of the intermediate as a white powder.

The reaction of the intermediate (13.4 mmol, 4 g) with EDA (373.5 mmol, 25 ml, 27.9 eq.) at 60°C was stirred for 24 hours. After completion extraction with EtOAc and then purification with silica gel column chromatography eluting with MeOH:DCM:NH_4OH (1: 4: 0.2) afforded 2.9 g (67%) of **9** as a white powder, m.p. 77.7°C; $C_{32}H_{53}N_7O_5$: ^1H NMR (600 MHz, DMSO) δ 7.79 (s, 4H, H-6'), 7.24 (s, 5H, H-7'), 6.93 (t, *J* = 7.2 Hz, 2H, H-8'), 3.30 (dd, *J* = 11.6, 5.6 Hz, 3H, H-2'), 2.71 (t, *J* = 6.0 Hz, 2H, H-1'). ^{13}C NMR (151 MHz, DMSO) δ 165.81 (C-4'), 164.00 (C-3'), 140.38 (C-5'), 128.28 (C-7'), 121.40 (C-8'), 119.77 (C-6'), 43.85 (C-2'), 41.25 (C-1'). COSY & HSQC (See annexure B). HRAPCI-MS *m/z* $[M+H]^+$: 322.1754 (Calcd. for $C_{32}H_{54}N_7O_5$: 322.1802).

5.5.4.3.7 2-N-(2-aminoethyl)-4-N-{2-[bis(propan-2-yl)amino]ethyl}-6-N-(4-methoxyphenyl)-1,3,5-triazine-2,4,6-triamine, 10

A solution of **2** (7.3 mmol, 2 g), Na_2CO_3 (14.6 mmol, 4.1 g, 2 eq.) and 2-(diisopropylamino)ethylamine (7.3 mmol, 1.2 ml, 1 eq.) in acetone (40 ml) was allowed to stir for 1 hour in an ice bath, and then 4 hours at room temperature followed by extraction with EtOAc. Purification by silica gel column chromatography eluting with MeOH:DCM (7:3) afforded 2.5 g of the intermediate as a brown powder.

The reaction of the intermediate (5.2 mmol, 2 g) with EDA (448.3 mmol, 30 ml, 86.2 eq.) at 61°C was stirred for 4 hours. After completion extraction with EtOAc and then purification with silica gel column chromatography eluting with MeOH:DCM:NH_4OH (1:4:0.5) afforded 2.1 g (100%) of **10** as a white powder, m.p. 68.3°C; $C_{20}H_{34}N_8O$: ^1H NMR (600 MHz, $CDCl_3$) δ 7.45 (s, 2H, H-7'), 6.77 (d, *J* = 8.5 Hz, 2H, H-8'), 3.81 – 3.65 (m, 3H, H-10'), 3.40 (d, *J* = 50.5 Hz, 4H, H-2', H-11'), 3.12 – 2.85 (m, 4H, H-12', H-13'), 2.62 (s, 2H, 1'), 1.01 (d, *J* = 6.3 Hz, 12H,

14'). ^{13}C NMR (151 MHz, $CDCl_3$) δ 165.85 (C-3', C-4'), 164.07 (C-5'), 155.14 (C-9'), 132.53 (C-6'), 121.61 (C-7'), 114.01 (C-8'), 55.23 (C-10'), 49.24 (C-13'), 44.51 (C-1'), 41.18 (C-11', C-12'), 40.12 (C-2'), 20.12 (C-14'). COSY & HSQC (See annexure B). HRAPCI-MS *m/z* $[M+H]^+$: 403.2908 (Calcd. for $C_{20}H_{35}N_8O$: 403.2956).

5.5.4.4 Artemisinin-triazine hybrids

The DHA (**1**) and triazine intermediates (**4** - **10**) together with K_2CO_3 were dissolved in DMF. The resulting mixture was heated in a microwave reactor in bursts of 60 W and 50°C for 4 min at a time followed by cooling to 0°C (Scheme 4). This sequence was repeated until the reaction was complete. Removal of the solvent *in vacuo*, dissolution of the residue in EtOAc, washing with H_2O, drying of the organic phase over $MgSO_4$ then concentration *in vacuo* resulted in an oil. The purification by silica gel column chromatography eluting with various ratios of MeOH, DCM, EtOAc, PE and NH_4OH afforded the free base target compounds, which were later converted into oxalate salts.

5.5.4.4.1 (2-{[bis(piperidin-1-yl)-1,3,5-triazin-2-yl]amino}ethyl)(2{[(1S,5R,9R,10S,13R)-1,5,9-trimethyl-11,14,15,16-tetraoxatetracyclo[10.3.1.0^{4,13}.0^{8,13}]hexadecan-10-yl]oxy}ethyl)amine, 11

The reaction between **1** (2.9 mmol, 1.1 g), K_2CO_3 (5.8 mmol, 0.786 g, 2 eq.) and **4** (2.9 mmol, 0.87 g, 1 eq.) in DMF (25 ml) followed by successive purification with silica gel column chromatography eluting with MeOH:DCM (1:4) then MeOH after which the conversion of the resulting oil into the salt afforded 0.4 g (19.5 %) of **11** as a white powder, m.p. 152.2°C; $C_{32}H_{53}N_7O_5$: ^{1}H NMR (600 MHz, $CDCl_3$) δ 5.32 (s, 1H, H-12), 4.73 (d, *J* = 3.2 Hz, 1H, H-10), 4.11 – 3.98 (m, 1H, H-aα), 3.81 – 3.56 (m, 11H, H-aβ, H-2', H-5'), 3.35 – 3.17 (m, 4H, H-1', H-b), 2.61 – 2.48 (m, 1H, H-9), 2.30 (td, *J* = 14.2, 3.7 Hz, 1H, H-4α), 2.01 – 1.90 (m, 1H, H-4β), 1.86 – 1.75 (m, 1H, H-5α), 1.71 – 1.48 (m, 15H. H-8α, H-8β, H-6', H-7', 7β), 1.48 – 1.22 (m, 7H, H-8a, H-5β, H-14, H-6), 1.17 (td, *J* = 11.4, 6.8 Hz, 1H, H-5a), 0.98 – 0.71 (m, 7H, H-7α, H-15, H-16). ^{13}C NMR (151 MHz, $CDCl_3$) δ 165.86 (Oxalic acid, C-3', C-4'), 103.94 (C-3), 102.14 (C-10), 87.84 (C-12), 81.19 (C-12a), 63.73 (C-a), 52.29 (C-5a), 47.65 (C-1', C-b), 44.31 (C-8a), 44.29 (C-5'), 37.78 (C-2'), 37.13 (C-6), 36.21 (C-4), 34.42 (C-7), 30.44 (C-9), 26.20 (C-6', C-14), 25.49 (C-7'), 24.41 (C-5), 24.19 (C-8), 20.11 (C-15), 12.97 (C-16). COSY & HSQC (See annexure B). HRESI-MS *m/z* $[M+H]^+$: 616.4182 (Calcd. for $C_{32}H_{54}N_7O_5$: 616.4208).

5.5.4.4.2 2-N-(4-methoxyphenyl)-6-(piperidin-1-yl)-4-N-{2-[(2-{[(1S,5R,9R,10S,13R)-1,5,9-trimethyl-11,14,15,16-tetraoxatetracyclo[10.3.1.0^{4,13}.0^{8,13}]hexadecan-10-yl]oxy}ethyl)amino]ethyl}-1,3,5-triazine-2,4-diamine, 12

The reaction between **1** (5.8 mmol, 2.28 g), K_2CO_3 (11.6 mmol, 1.6 g, 2 eq.) and **5** (5.8 mmol, 2 g, 1 eq.) in DMF (50 ml) followed by successive purification with silica gel column chromatography eluting with MeOH:EtOAc:NH_4OH (1:14:0.5) then MeOH:EtOAc:NH_4OH (19:1:0.5) after which the conversion of the resulting oil into the salt afforded 0.5 g (12 %) of **12** as a white powder, m.p. 151.5°C; $C_{34}H_{51}N_7O_6$: ^{1}H NMR (600 MHz, $CDCl_3$) δ 7.45 (d, *J* = 8.2 Hz, 2H, H-10'), 6.75 (d, *J* = 8.5 Hz, 2H, H-11'), 5.40 – 5.26 (m, 1H, H-12), 4.75 (t, *J* = 18.4 Hz, 1H, H-10), 4.18 – 4.00 (m, 1H, H-aα), 3.92 – 3.54 (m, 10H, H-aβ, H-2', H-13', H-6'), 3.45 – 3.11 (m, 4H, H-1', H-b), 2.66 – 2.46 (m, 1H, H-9), 2.30 (ddd, *J* = 18.4, 17.2, 8.9 Hz, 1H, 4α), 2.07 – 1.88 (m, 1H, H-4β), 1.86 – 1.74 (m, 1H, H-5α), 1.60 (d, *J* = 47.4 Hz, 9H, H-8', H-7', H-8α, H-7β, H-8β), 1.47 – 1.06 (m, 8H, H-6, H-5β, H-8a, H-14, H-5a), 0.96 – 0.63 (m, 7H, H-7α, H-15, H-16). ^{13}C NMR (151 MHz, $CDCl_3$) δ 164.91 (C-5'), 161.68 (C-3'), 156.67 (C-4'), 155.98 (Oxalic acid), 153.61 (C-12'), 129.89 (C-9'), 123.16 (C-10'), 113.70 (C-11'), 103.93 (C-3), 102.10 (C-10), 87.98 (C-12), 81.03 (C-12a), 63.89 (C-a), 55.45 (C-13'), 52.44 (C-5a), 47.58 (C-1', C-b), 45.19 (C-6'), 44.18 (C-8a), 37.76 (C-6), 37.15 (C-2'), 36.35 (C-4), 34.12 (C-7), 30.53 (C-9), 26.17 (C-14), 25.65 (C-7'), 24.29(C-5, C-8'), 24.14 (C-8), 20.25 (C-15), 12.89 (C-16). COSY & HSQC (See annexure B). HRESI-MS *m/z* $[M+H]^+$: 654.3970 (Calcd. for $C_{34}H_{52}N_7O_6$: 654.4001).

5.5.4.4.3 (2-{[bis(morpholin-4-yl)-1,3,5-triazin-2-yl]amino}ethyl)(2-{[(1S,5R,9R,10S,13R)-1,5,9-trimethyl-11,14,15,16-tetraoxatetracyclo[10.3.1.0^{4,13}.0^{8,13}]hexadecan-10-yl]oxy}ethyl)amine, 13

The reaction between **1** (1.6 mmol, 0.632 g), K_2CO_3 (3.2 mmol, 0.45 g, 2 eq.) and **6** (1.6 mmol, 0.5 g, 1 eq.) in DMF (25 ml) followed by purification with silica gel column chromatography eluting with MeOH:DCM (1:9) after which the conversion of the resulting oil into the salt afforded 0.3 g (28 %) of **13** as a white powder, m.p. 162.1°C; $C_{30}H_{49}N_7O_7$: ^{1}H NMR (600 MHz, DMSO) δ 5.31 (d, *J* = 37.4 Hz, 1H, H-12), 4.69 (d, *J* = 3.3 Hz, 1H, H-10), 3.85 (dt, *J* = 10.6, 5.2 Hz, 1H, H-aα), 3.71 – 3.41 (m, 21H, H-aβ, H-5', H-6', H-2'), 3.15 – 2.85 (m, 4H, H-1', H-b), 2.42 – 2.30 (m, 1H, H-9), 2.16 (td, *J* = 14.1, 3.7 Hz, 1H, H-4α), 2.08 – 1.91 (m, 1H, H-4β), 1.84 – 1.71 (m, 1H, 5α), 1.68 – 1.53 (m, 2H, H-8α, H-8β), 1.48 (dd, *J* = 12.8, 2.7 Hz, 1H, H-7β), 1.40 – 1.19 (m, 6H, H-5β, H-6, H-8a, H-14), 1.17 – 1.02 (m, 1H, H-5a), 0.82 (ddd, *J* = 32.2, 20.8, 6.4 Hz, 7H, H-7α, H-15, H-16). ^{13}C NMR (151 MHz, DMSO) δ 165.72 (Oxalic acid, C-3'), 164.54 (C-4'), 103.28 (C-3), 100.91 (C-10), 87.11 (C-12), 80.38 (C-12a),

66.04 (C-6'), 64.23 (C-a), 51.94 (C-5a), 47.36 (C-1'), 46.39 (C-b), 43.72 (C-8a), 43.18 (C-5'), 37.26 (C-2'), 36.42 (C-6), 36.10 (C-4), 34.10 (C-7), 30.06 (C-9), 25.47 (C-14), 23.98 (C-5), 23.64 (C-8), 20.09 (C-15), 12.55 (C-16). COSY & HSQC (See annexure B). HRESI-MS *m/z* $[M+H]^+$: 620.3758 (Calcd. for $C_{30}H_{50}N_7O_7$: 620.3794).

5.5.4.4.4 2-N-(4-methoxyphenyl)-6-(morpholin-4-yl)-4-N-{2-[(2-{[(1S,5R,9R,10S,13R)-1,5,9-trimethyl-11,14,15,16-tetraoxatetracyclo[10.3.1.0^{4,13}.0^{8,13}]hexadecan-10-yl]oxy}ethyl)amino]ethyl}-1,3,5-triazine-2,4-diamine, 14

The reaction between **1** (2.9 mmol, 1.14 g), K_2CO_3 (5.8 mmol, 0.8 g, 2 eq.) and **7** (2.9 mmol, 1 g, 1 eq.) in DMF (50 ml) followed by successive purification with silica gel column chromatography eluting with MeOH:EtOAc:PE:NH_4OH (1:10:4:0.5) then MeOH:EtOAc:NH_4OH (1:19:0.5) after which the conversion of the resulting oil into the salt afforded 0.3 g (13 %) of **14** as a white powder, m.p. 140.9°C; $C_{33}H_{49}N_7O_7$: ^{1}H NMR (600 MHz, $CDCl_3$) δ 7.42 (d, *J* = 7.7 Hz, 2H, H-7'), 6.81 (dd, *J* = 47.4, 8.4 Hz, 2H. H-8'), 5.37 (d, *J* = 22.7 Hz, 1H, H-12), 4.76 (d, *J* = 27.2 Hz, 1H, H-10), 4.09 (s, 1H, H-aα), 3.96 – 3.52 (m, 14H, H-aβ, H-2', H-12', H-10', H-11'), 3.42 (dd, *J* = 37.3, 30.1 Hz, 4H, H-1', H-b), 2.57 (s, 1H, H-9), 2.29 (d, *J* = 12.5 Hz, 1H, H-4α), 1.96 (d, *J* = 26.3 Hz, 2H, H-4β), 1.81 (s, 1H, H-5α), 1.72 – 1.49 (m, 3H, H-8α, H-8β, H-7β), 1.28 (ddd, *J* = 32.8, 24.1, 6.1 Hz, 8H, H-6, H-5β, H-8a, H-14, H-5a), 0.86 (dd, *J* = 29.8, 5.7 Hz, 7H, H-7α, H-15, H-16). ^{13}C NMR (151 MHz, $CDCl_3$) δ 163.43 (Oxalic acid), 161.85 (C-4'), 156.92 (C-3'), 156.00 (C-5'), 153.79 (C-9'), 129.18 (C-6'), 123.60 (C-7'), 113.59 (C-8'), 104.15 (C-3), 102.36 (C-10), 87.63 (C-12), 80.47 (C-12a), 66.33 (C-12'), 63.86 (C-a), 55.25 (C-10'), 52.62 (C-5a), 47.77 (C-1'), 47.05 (C-b), 44.24 (C-8a), 44.06 (C-11'), 37.89 (C-2'), 36.95 (C-6), 36.39 (C-4), 34.13 (C-7), 30.41 (C-9), 25.96 (C-14), 24.41 (C-5), 24.01 (C-8), 19.93 (C-15), 12.81 (C-16). COSY & HSQC (See annexure B). HRESI-MS *m/z* $[M+H]^+$: 656.3752 (Calcd. for $C_{33}H_{50}N_7O_7$: 656.3797).

5.5.4.4.5 2-N-(4-methoxyphenyl)-4-N-phenyl-6-N-{2-[(2-{[(1S,5R,9R,10S,13R)-1,5,9-trimethyl-11,14,15,16-tetraoxatetracyclo[10.3.1.0^{4,13}.0^{8,13}]hexadecan-10-yl]oxy}ethyl)amino]ethyl}-1,3,5-triazine-2,4,6-triamine, 15

The reaction between **1** (5.7 mmol, 2.2 g), K_2CO_3 (11.4 mmol, 1.57 g, 2 eq.) and **8** (5.7 mmol, 2 g, 1 eq.) in DMF (50 ml) followed by successive purification with silica gel column chromatography eluting with MeOH:DCM (1:4) then MeOH:DCM:NH_4OH (1:9:0.5) after which the conversion of the resulting oil into the salt afforded 0.6 g (14 %) of **15** as a white powder, m.p. 156.0°C; $C_{35}H_{47}N_7O_6$: ^{1}H NMR (600 MHz, DMSO) δ 7.67 (d, *J* = 85.8 Hz, 5H, H-7', H-11'), 7.23 (d, *J* = 6.5 Hz, 2H, H-8'), 6.98 – 6.78 (m, 3H, H-9', H-12'), 5.34 (s, 1H, H-12), 4.68 (d, *J* =

2.4 Hz, 1H, H-10), 3.90 (d, *J* = 4.9 Hz, 1H, H-aα), 3.71 (s, 3H, H-14'), 3.57 (m, H-2', H-aβ, H_2O), 3.25 – 3.18 (m, 4H, H-1', H-b), 2.36 (dd, *J* = 7.2, 3.9 Hz, 1H, H-9), 2.14 (td, *J* = 14.2, 3.7 Hz, 1H, H-4α), 1.95 (d, *J* = 13.7 Hz, 1H, H-4β), 1.82 – 1.68 (m, 1H, H-5α), 1.63 – 1.52 (m, 2H, H-8α, H-8β), 1.44 (d, *J* = 10.5 Hz, 1H, H-7β), 1.36 – 1.17 (m, 7H, H-6, H-5β, H-8a, H-14), 1.12 – 1.03 (m, 1H, H-5a), 0.83 – 0.67 (m, 8H, H-7α, H-15, H-16). ^{13}C NMR (151 MHz, $CDCl_3$) δ 165.89 (C-3'), 164.54 (Oxalic acid), 163.92 (C-4', C-5'), 154.57 (C-13'), 128.38 (C-8'), 121.95 (C-11'), 121.67 (C-9'), 119.88 (C-7'), 113.64 (C-12'), 103.14 (C-3), 101.14 (C-10), 87.27 (C-12), 80.32 (C-12a), 63.36 (C-a), 55.09 (C-14'), 51.95 (C-5a), 46.14 (C-1', C-b), 43.74 (C-8a), 36.98 (C-2'), 36.35 (C-6), 35.85 (C-4), 34.08 (C-7), 30.28 (C-9), 25.64 (C-14), 24.27 (C-5), 23.84 (C-8), 20.04 (C-15), 12.61 (C-16). COSY & HSQC (See annexure B). HRESI-MS *m/z* $[M+H]^+$: 662.3656 (Calcd. for $C_{35}H_{48}N_7O_6$: 662.3688).

5.5.4.4.6 2-N,4-N-diphenyl-6-N-{2-[(2-{[(1S,5R,9R,10S,13R)-1,5,9-trimethyl-11,14,15,16-tetraoxatetracyclo[10.3.1.0^{4,13}.0^{8,13}]hexadecan-10-yl]oxy}ethyl)amino]ethyl}-1,3,5-triazine-2,4,6-triamine, 16

The reaction between **1** (2.3 mmol, 0.9 g), K_2CO_3 (4.6 mmol, 0.63 g, 2 eq.) and **9** (2.3 mmol, 0.724 g, 1 eq.) in DMF (25 ml) followed by successive purification with silica gel column chromatography eluting with MeOH:EtOAc:NH_4OH (1:29:0.5) then MeOH:EtOAc:NH_4OH (1:9:0.5) after which the conversion of the resulting oil into the salt afforded 0.2 g (13.4 %) of **16** as a white powder, m.p. 136.8°C; $C_{34}H_{45}N_7O_5$: ^{1}H NMR (600 MHz, DMSO) δ 7.76 (s, 4H, H-6'), 7.25 (dd, *J* = 13.4, 6.9 Hz, 6H, H-7',NH), 6.95 (d, *J* = 6.4 Hz, 2H, H-8'), 5.35 (s, 1H, H-12), 4.69 (d, *J* = 3.2 Hz, 1H, H-10), 3.96 – 3.87 (m, 1H, H-aα), 3.71 – 3.49 (m, 3H, H-aβ, H-2'), 3.25 – 3.13 (m, 4H, H-1', H-b), 2.40 – 2.30 (m, 1H. H-9), 2.14 (td, *J* = 14.2, 3.8 Hz, 1H, H-4α), 1.99 – 1.90 (m, 1H, H-4β), 1.79 – 1.68 (m, 2H, H-5α), 1.60 (dt, *J* = 26.0, 13.0 Hz, 2H, H-8α, H-8β), 1.45 (d, *J* = 10.2 Hz, 1H, 7β), 1.37 – 1.17 (m, 6H, H-6, H-8a, H-5β, H-14), 1.09 (dt, *J* = 19.1, 6.8 Hz, 3H, H-5a, Diethyl ether's CH_3), 0.91 – 0.59 (m, 8H, H-7α, H-15, H-16). ^{13}C NMR (151 MHz, $CDCl_3$) δ 165.73 (C-3'), 163.89 (C-4'), 163.06 (Oxalic acid), 140.11 (C-5'), 128.39 (C-7'), 121.83 (C-8'), 120.05 (C-6'), 103.27 (C-3), 101.03 (C-10), 86.96 (C-12), 80.54 (C-12a), 63.35 (C-a), 51.94 (C-5a), 46.01 (C-1', C-b), 43.69 (C-8a), 36.78 (C-2'), 36.47 (C-6), 35.94 (C-4), 34.10 (C-7), 30.22 (C-9), 25.61 (C-14), 24.29 (C-5), 23.80 (C-8), 20.11 (C-15), 12.55 (C-16). COSY & HSQC (See annexure B). HRESI-MS *m/z* $[M+H]^+$: 632.3540 (Calcd. for $C_{34}H_{46}N_7O_5$: 632.3582).

5.5.4.4.7 2-N-{2-[bis(propan-2-yl)amino]ethyl}-4-N-(4-methoxyphenyl)-6-N-{2-[(2-{[(1S,5R,9R,10S,13R)-1,5,9-trimethyl-11,14,15,16-tetraoxatetracyclo[10.3.1.$0^{4,13}$.$0^{8,13}$]hexadecan-10-yl]oxy}ethyl)amino]ethyl}-1,3,5-triazine-2,4,6-triamine, 17

The reaction between **1** (5.0 mmol, 1.94 g), K_2CO_3 (9.94 mmol, 1.37 g, 2 eq.) and **10** (5.0 mmol, 2.0 g, 1 eq.) in DMF (50 ml) followed by successive purification with silica gel column chromatography eluting with MeOH:DCM:NH_4OH (1:9:0.5) then MeOH:EtOAc:NH_4OH (1:14:0.5) after which the conversion of the resulting oil into the salt afforded 0.5 g (11 %) of **17** as a white powder, m.p. 152.0°C; $C_{37}H_{60}N_8O_6$: ^{1}H NMR (600 MHz, $CDCl_3$) δ 7.45 (s, 2H, H-7'), 6.81 (d, *J* = 8.6 Hz, 2H, H-8'), 5.36 (s, 1H, H-12), 4.78 (t, *J* = 13.1 Hz, 1H, H-10), 3.95 – 3.86 (m, 1H, H-aα), 3.76 (s, 3H, H-10'), 3.48 (dt, *J* = 48.9, 21.9 Hz, 5H, H-aβ, H-2', H-11'), 3.09 (s, 2H, H-13'), 2.80 (dd, *J* = 11.9, 6.6 Hz, 4H, H-b, H-1'), 2.68 (s, 2H, H-12'), 2.61 – 2.55 (m, 1H, H-9), 2.32 (td, *J* = 14.1, 3.9 Hz, 1H, H-4α), 2.02 – 1.96 (m, 1H, H-4β), 1.84 (ddd, *J* = 13.6, 6.4, 3.3 Hz, 1H, H-5α), 1.67 (dd, *J* = 25.8, 10.8 Hz, 2H, H-8α, H-8β), 1.60 – 1.54 (m, 1H, H-7β), 1.47 – 1.36 (m, 5H, H-8a, H-5β, H-14), 1.30 – 1.19 (m, 3H, H-5a, H-6), 1.06 (s, 14H, H-14'), 0.90 – 0.79 (m, 7H, H-7α, H-15, H-16). ^{13}C NMR (151 MHz, $CDCl_3$) δ 155.33 (C-9'), 132.41 (C-6'), 121.81 (C-7'), 113.89 (C-8'), 104.15 (C-3), 102.09 (C-10), 87.76 (C-12), 81.01 (C-12a), 67.56 (C-a), 55.47 (C-10'), 52.41 (C-5a), 48.75 (C-b), 48.54 (C-1'), 44.32 (C-8a), 44.19 (C-12'), 40.30 (C-2', C-11'), 37.41(C-6), 36.26 (C-4), 34.43 (C-7), 30.72 (C-9), 26.01 (C-14), 24.61 (C-5), 24.48 (C-8), 20.24 (C-15, C-14'), 12.87 (C-16). COSY & HSQC (See annexure B). HRESI-MS *m/z* $[M+H]^+$: 713.469 (Calcd. for $C_{37}H_{61}N_8O_6$: 713.444).

Conflict of interest

The authors declare that they have no conflict of interest to disclose.

Acknowledgements

We thank the National Research Foundation (NRF) and the North-West University (NWU) for financial support. We would also like to thank the German Academic Exchange Service (DAAD) for a study bursary to TT Cloete. In addition we would like to thank André Joubert for his help with the NMR acquisition and Marelize Ferreira and Johan Jordaan for assisting with the MS attainment and interpretation.

References

1. WHO. 2011. World Malaria Report 2011. [Web:] http://www.who.int/ malaria/world_malaria_report_2011/9789241564403_eng.pdf [Date used: 7 February 2012].
2. Schlitzer, M. *Arch. Pharm. Chem. Life. Sci*. **2008**, *341*, 149.
3. Petersen, I.; Eastman, R.; Lanzer, M. *FEBS Lett*. **2011**, *585*, 1551.
4. Posner, G.H.; Paik, I.H.; Chang, W.; Borstnik, K.; Sinishtaj, S.; Rosenthal, A.S.; Shapiro, T.A. *J. Med. Chem*. **2007**, *50*, 2516.
5. Galal, A.M.; Gul, W.; Slade, D.; Ross, S.A.; Feng, S.; Hollingshead, M.G.; Alley, M.C.; Kaur, G.; ElSohly, M.A. *Bioorg. Med. Chem.* **2009**, *17*, 741.
6. Na-Bangchang, K.; Karbwang, J. *Fundam. Clin. Pharmacol*. **2009**, *23*, 387.
7. WHO, Guidelines for the treatment of malaria 2nd ed. **2010**, pp 1 -194.
8. Dondorp, A. M.; Nosten, F.; Yi, P.; Das, D.; Phyo, A. P.; Tarning, J.; Lwin, K. M.; Ariey, F.; Hanpithakong, W.; Lee, S. J.; Ringwald, P.; Silamut, K.; Imwong, M.; Chotivanich, K.; Lim, P.; Herdman, T.; An, S. S.; Yeung, S.; Singhasivanon, P.; Day, N. P. J.; Lindegardh, N.; Socheat, D.; White, N. J. *N. Engl. J. Med*. **2009**, *361*, 455.
9. Meunier, B. *Acc. Chem. Res*. **2008**, *41*, 69.
10. Agarwal, A.; Srivastava, K.; Purib, S.K.; Chauhan, P.M.S. *Bioorg. Med. Chem. Lett*. **2005**,*15*, 531.
11. Zhou, Y.; Sun, Z.; Froelich, J.M.; Hermann, T.; Wall, D. *Bioorg. Med. Chem. Lett*. **2006**, *16*, 5451.
12. Kinyanjui, S.M.; Mberu, E.K.; Winstanley, P.A.; Jacobus, D.P.; Watkins, W.M. *Am. J. Trop. Med. Hyg*. **1999**, *6*, 943.
13. Manohar, S.; Khan, S.I.; Rawat, D.S. *Bioorg. Med. Chem. Lett*. **2010**, *20*, 322.
14. Kumar, A.; Srivastava, K.; Kumar, S.R.; Puri, S.K.; Chauhan, P.M.S. *Bioorg. Med. Chem. Lett*. **2009**, *19*, 6996.
15. Hawser, S.; Lociuro, S.; Islam. K. *Biochem. Pharmacol*. **2006**, *71*, 941.
16. Olliaro, P. *Pharmacol. Ther*. **2001**, *89*, 207.
17. Uhlemann, A.C., Krishna, S. *Springer verlag*. **2005**, *295*, 39.
18. Yuthavong, Y. *Microb. Infect*. **2002**, *4*, 175.
19. Li, Y.; Zhu, Y.; Jiang, H.; Pan, J.; Wu, G.; Wu, J.; Shi, Y.; Yang, J.; Wu, B. *J. Med. Chem*. **2000**, *43*, 1635.
20. Cloete, T.T.; Breytenbach, J.W.; de Kock, C.; Smith, P.J.; Breytenbach, J.C.; N'Da, D.D. *Bioorg. Med. Chem*. **2012**, *20*, 4701.
21. Haynes, R.K.; Chan, H.W.; Cheung, M.K.; Lam, W.L.; Soo, M.K.; Tsang, H.W.; Voerste, A.; Williams, I.D. *Eur. J. Org. Chem*. **2002**, *1*, 113.

22. Lombard, M.C.; Fernandes, M.A.; Breytenbach, J.C.; N'Da, D.D. *Acta Crys. E*. **2010**, *66*, 2182.
23. Torok, S. D.; Ziffer, H. Tetrahedron Lett. 1995, 6, 829
24. Guan, J.; Wang, X.; Smith, K.; Ager, A.; Gettayacamin, M.; Kyle, D.E.; Milhous, W.K.; Kozar, M.P.; Magill, A.J.; Lin, A.J. *J. Med. Chem*. **2007**, *50*, 6226.
25. Woodrow, C.J.; Haynes, R.K.; Krishna, S. *Postgrad. Med. J*. **2005**, *81*, 71.
26. Grace, J.M.; Aguilar, A.J.; Trotman, K.M.; Brewer, T.G. *Drug Metab. Dispos*. **1997**, *26*, 313.
27. Karbwang, J.; Na-Bangchang, K.; Congpuong, K.; Molunto, P.; Thanavibul, A*. Eur. J. Clin. Pharmacol*. **1997**, *52*, 307.
28. Advanced Chemistry Inc. ACD/ChemSketch, **2000**, version 4.54.http://www.acdlabs.com
29. Hindley, S.; Ward, S.A.; Storr, R.C.; Searle, N.L.; Bray, P.G.; Park, K.; Davies, J.; O'Niel, P.M. *J. Med. Chem*. **2002**, *45*, 1052.
30. Egan, T.J. *Targets*. **2003**, *2*, 115.
31. Lipinski, C.A.; Lombardo, F.; Dominy, B.W.; Feeney, P.K. *Adv. Drug. Deliver. Rev*. **2001**, *46*, 3.
32. Caron, G.; Raymond, F.; Carrupt, P.A.; Girault, H.H.; Testa, B. *Pharm. Sci. Technol. Today*. **1999**, *2*, 327.
33. Bigucci, F.; Kamsu-Kom, T.; Cholet, C.; Besnard, M.; Bonnet-Delpon, D.; Ponchel, G.J. *Pharm. Pharmacol*. **2008**, *60*, 163.
34. Trager, W.; Jensen, J.B. *Science*. **1976**, *193*, 673.
35. Smilkstein, M.; Sriwilaijaroen, N.; Kelly, J.X.; Wilairat, P.; Riscoe, M. *Antimicrob. Agents Chemother*. **2004**, *48*, 1803.
36. R Development Core Team, R. A language and environment for statistical computing, R Foundation for Statistical Computing. **2011**, Vienna, Austria, http://www.R-project.org.
37. Ritz, C.; Streibig, J.C.; Statist. J. *Software*. **2005**, *12*, 1.