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Original article

Synthesis, *in vitro* antiplasmodial activity and cytotoxicity of a series of artemisinin–triazine hybrids and hybrid-dimers

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ABSTRACT

A series of artemisinin–triazine hybrids and hybrid-dimers were synthesized and their *in vitro* antimalarial activity against the chloroquine sensitive (CQS), the gametocytocidal (NF54) and the choroquine resistant (CQR) Dd2 strains of *Plasmodium falciparum* determined, while their toxicity against CHO cells were also established. These compounds were prepared by linking artemisinin and triazine pharmacophores through nucleophilic substitution, using conventional and microwave assisted methods. These hybrids and hybrid-dimers were all found to be active against all three *Plasmodium* strains, with the *p*-anisidino-substituted triazine hybrid-dimer **22** being the most active of all. It showed good anti-gametocytocidal activity against the NF54 strain, with a 50% inhibitory concentration value in the nanomolar range, while having a potency comparable to that of artesunate against the Dd2 strain. This hybrid-dimer further demonstrated selective toxicity towards the parasitic cells. This dimer hence showed the necessary potential as candidate for further investigation in the search for malaria transmission blocking drugs so desperately needed.

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1. Introduction

Malaria has been a major parasitic disease of man since antiquity and continues to pose an enormous health risk today. Despite our increased knowledge of this disease, currently around 6.6 billion people are at risk of contracting malaria, with an estimated 655,000 people dying from this disease, annually [1]. The five species of the *Plasmodium* (*P*) parasite that can 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, comprising artemisinin (ART), dihydroartemisinin (DHA), artemether (ArM) and artesunate (AS) [2]. Unfortunately, due to the parasites' remarkable abilities to acquire resistance against most of these drugs, only the artemisinin class of compounds remains a viable first line treatment option for uncomplicated *P. falciparum* malaria in most of the areas afflicted by the disease [3].

Although the mechanism of action of the artemisinins is still under investigation, these compounds have proven 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 actions against the gametocyte forms of the parasite [4]. Despite these advantages, known disadvantages include their poor water (ART, DHA and ArM) and oil solubilities (AS), biological short half-lives and high parasite recrudescence after treatment [5].

In an effort to enhance the efficiency of antimalarial drugs and to counter the spread of resistance, the artemisinin class of compounds is 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 destroying the parasite and delaying the onset of resistance [6]. The World Health Organization (WHO) now recommends using an ACT as the first line of 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

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artemisinin class of compounds being the foundation on which treatment regimens are currently built, the loss of this important class of drugs 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 advantages of these types of molecules, such as 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, *e.g.* increased solubility [9].

In this study, hybrid molecules, consisting of artemisinin and triazine moieties were 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). Substituted, this moiety has been found to have relatively good activity against malaria parasites and enzymes, both on its own and attached to other pharmacophores [10–14].

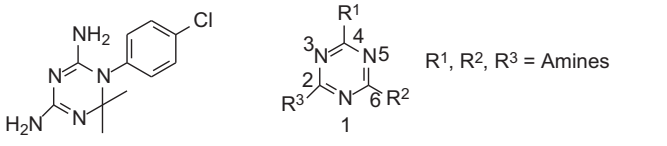
The folate biosynthetic pathway in the parasite consists of a process in which six enzymes are involved in the conversion of guanosine triphosphate (GTP) into tetrahydrofolic acid (THF), which is of vital importance in the synthesis of purines, thymidine and some amino acids [15]. The folate antagonists, also known as antifolates, can be divided into two classes, *viz*. type 1 and type 2 antifolates. The type 1 antifolates include the 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 comprise pyrimethamine (PM), the quinazolines, biguanides and triazine metabolites. These antifolates inhibit dihydrofolate reductase (DHFR), preventing the nicotinamide adenine dinucleotide phosphate (NADPH) dependent reduction of dihydrofolate (DHF) into THF [16]. Not long after the introduction of these antimalarials onto the market, worldwide resistance against this class spread rapidly, making them obsolete in most areas afflicted by malaria [17]. The combination of both types 1 and 2 antifolates, *e.g.* sulfadoxine and pyrimethamine (SP), had proven a synergistic antimalarial effect, but resistance against these combinations also developed quickly, preventing them from being used on a significant scale against malaria [18].

The compounds described in this article contain the artemisinin and 1,3,5-triazine pharmacophores and were designed for activity against both the sensitive and resistant strains of *P. falciparum*. Reported herein are their synthesis, *in vitro* antimalarial activity against various strains of *P. falciparum* and their cytotoxicity.

2. Results

2.1. Chemistry

Condensation of dihydroartemisinin with 2-bromoethanol produced the DHA–ethyl bromide derivative **1** in good yield, using a reported method (Fig. 2) [19,20]. The stereochemistry of this



Cycloguanil **2, 4, 6-Trisubstituted-1, 3, 5-trazine moiety**

Fig. 1. Structures of cycloguanil and the 2,4,6-tri-substituted-1,3,5-triazine moiety.

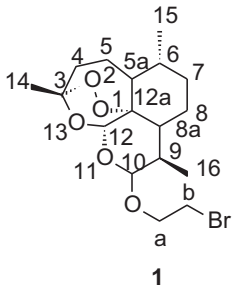


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Fig. 2. Structure of 2-bromo-(10β-dihydroartemisinoxy)ethane **1**.

compound had previously been determined in our research group by X-ray analysis [21]. This intermediate and the final hybrids and hybrid-dimers were all in the 10β form, as confirmed by the small coupling constant, $J = 3.4$ Hz, between the H-10 and H-9 atoms [22].

The trichloro-substituted triazine, cyanuric chloride (CyCl) **2**, was then reacted with the relevant amine to produce the mono-substituted compounds **3** and **4**, with yields of 68% and 86%, respectively. Subsequently, either **2**, **3** or **4** was reacted with different amines, generating the di-substituted compounds, which upon reaction with ethylenediamine (EDA) resulted in the primary amine functionalised tri-substituted triazine intermediates **5**–**13**, with yields ranging between 49 and 90% (Scheme 1). The infrared (IR) spectra of these intermediates commonly showed absorption bands in the 3360–3250 cm^{-1} region, evident of the presence of both NH_2 and NH groups (Supplementary information 1).

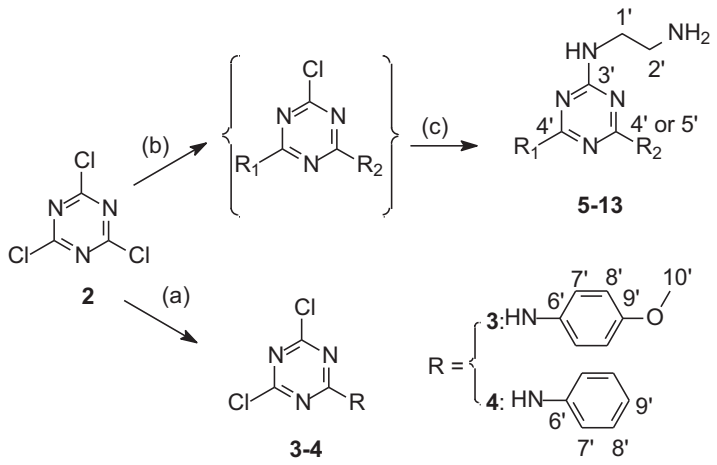
Finally, each intermediate was reacted with compound **1** through nucleophilic substitution, using microwave radiation to render a free base hybrid (**14**, **15**, **16**, **18**, **20**, **23**, and **25**) and/or a hybrid-dimer (**17**, **19**, **21**, **22**, **24**, and **26**) (Scheme 2), which for stability reasons were all converted into oxalate salts with yields in the 3–28% range. The IR spectra of the hybrids and hybrid-dimers displayed a shoulder in the 3460–3200 cm^{-1} region, assignable to vibrations of the secondary amine (NH) groups. A sharp band, corresponding with the vibrations of an O–O bond, was also present in the 2950–2830 cm^{-1} range, confirming the presence of the artemisinin moiety in these structures. A sharp band at ~1720 cm^{-1}, corresponding with the vibrations of the carboxylate C–O, also confirmed the presence of an oxalate group (Supplementary information 2).

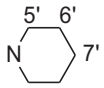
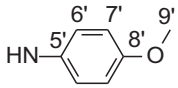
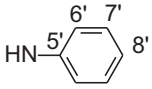
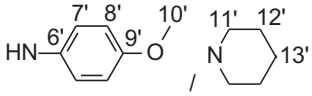
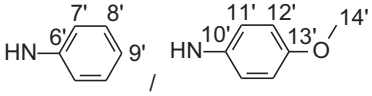
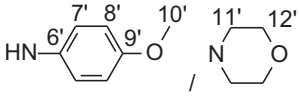
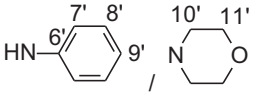
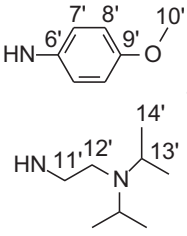
2.2. Biological activity

The antimalarial activities and cytotoxicities of the synthesized compounds are summarised in Table 1. All hybrids and hybrid-dimers were found to be less potent than both DHA and AS against the NF54 strain, although they were up to six thousand-fold more active than PM. Compared to chloroquine (CQ), only dimer **22** was at least twice as potent, while the remaining compounds were either less, or equally potent. No synthesized compound showed better activity than the equimolar combination of DHA and PM.

With regards to the Dd2 strain, the results were quite different. All of the hybrids and hybrid-dimers were more potent than both CQ and PM. More significantly, dimers **22**, **24** and **26** displayed potencies comparable to those of DHA, AS and the DHA:PM mixture (**M**). Furthermore, where both the hybrids and counterpart hybrid-dimers were isolated and screened, the dimer proved to be more active than its corresponding hybrid, irrespective of the strain being tested against.

The resistance index (RI) value of most hybrids and hybrid-dimers was found to be below 2, an indication of a moderate loss



Interm	R_1 / R_2	Interm	R_1 / R_2
5		**10**	
6		**11**	
7		**12**	
8		**13**	
9			

Scheme 1. Synthesis of intermediates **5**–**13**. Reagents and conditions: (a) appropriate amine, acetone, Na_2CO_3, 6 h, 0 °C; (b) appropriate amine, acetone, Na_2CO_3, 1–3 h, 0 °C; (c) 3–24 h, r.t.; (d) EDA, 3–24 h, 50–70 °C.

of activity against the CQR strain Dd2, compared to the CQS. This loss was, however, more significant for CQ, **M** and DHA with RI values of 22, 12.4 and 9.7, respectively. The synthesized compounds, together with the equimolar DHA:PM combination proved to be selectively more toxic to the parasitic cells in the presence of mammalian cells (SI > 50), with hybrid **19** (SI = 25) and hybrid-dimer **20** (SI = 34) being the least selective, while PM (SI = 8) was found to be noticeably toxic to the mammalian cells, but sparing the parasitic ones.

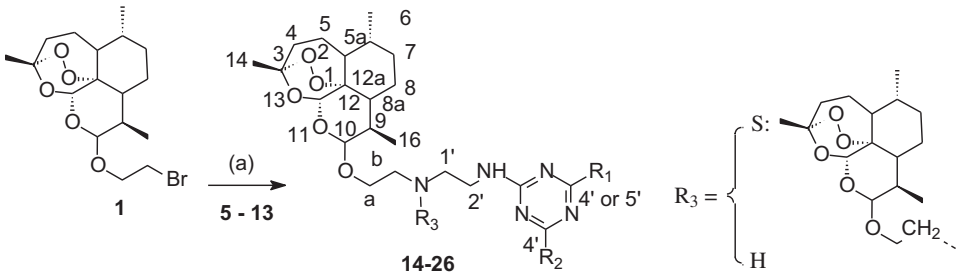
3. Discussion

3.1. Chemistry

The artemisinin-triazine hybrids and hybrid-dimers were synthesized using microwave radiation. The microwave reaction was carried out in a 250 mL open vessel round bottom flask, connected to a Liebig cooler, with a ramp and hold time of 1 and 4 min, respectively. The maximum allowed temperature and power settings were 50 °C and 60 W. The reaction was carried out by increasing the electrical power, until either the temperature or the hold time was reached. During each cycle, cooled air was pumped into the reactor, keeping the temperature as low as possible. The reaction vessel was then cooled to −8 °C in preparation for the next burst. These bursts were repeated 8 to 13 times, until thin layer chromatography (TLC) indicated completion of the reaction.

The peroxide bridge of artemisinin is reported to be prone to facile hydrolysis under basic or acidic conditions and can also be expected to be heat sensitive [23]. However, in this study, the spectroscopic data revealed no deviations from the expected values, confirming that this bridge had been preserved during microwave radiation, hence supporting previous findings [20].

In intermediates **5**–**13**, the secondary amine (NH) of the EDA linker was less nucleophilic as a result of the resonance of the already di-substituted triazine ring, making it unavailable for substitution reactions. Subsequently, only the terminal primary amine



Hybrid/Dimer.	R_1/R_2 of Intermediate	R_3	Hybrid/Dimer.	R_1/R_2 of Intermediate	R_3
14	**5**	H	**21**	**9**	S
15	**6**	H	**22**	**10**	S
16	**7**	H	**23**	**11**	H
17	**7**	S	**24**	**11**	S
18	**8**	H	**25**	**12**	H
19	**8**	S	**26**	**13**	S
20	**9**	H			

Scheme 2. Synthesis of artemisinin–triazine hybrids and hybrid-dimers **14**–**26**. Reagents and conditions: (a) DMF, K_2CO_3, microwave (60 W, 50 °C, 4 min); (b) 0 °C.

was involved in the substitution reactions, leading to the formation of the hybrids and/or hybrid-dimers. For stability and solubility reasons, the free base target compounds were converted into oxalate salts, with the unfortunate disadvantage of considerably reducing the overall yields.

Compound structures were confirmed using IR, high resolution mass spectrometry (HRMS), and both 1D (1H and ^{13}C nuclear magnetic resonance) and 2D (correlation spectroscopy (COSY) and heteronuclear single quantum coherence (HSQC)) techniques, due to their complex structures.

Table 1
IC_{50}-values of compounds tested *in vitro* for antiplasmodial activity against NF54 and Dd2 strains of *Plasmodium falciparum* and their cytotoxicity against CHO cells. Cells were incubated with compounds at various concentrations for 48 h; antimalarial activity and cytotoxicity were determined using parasite lactate dehydrogenase (pLDH) and MTT-assays, respectively.

Comp	Log P[c]	pK_a[c]	Activity, IC_{50} [nM (ng/ml) ± SD][a]		Resistance index	Cytotoxicity, IC_{50} [μM (μg/ml) ± SD][b]	Selectivity index
			NF54	Dd2	RI[d]	CHO	SI[e]
14	6.7	8.6	20.6 (12.7) ± 3.4	47.5 (29.3) ± 1.5	2.3	3.1 (1.9) ± 0.2	150
15	3.6	8.6	19.3 (12.0) ± 7.9	85.0 (52.7) ± 22.9	4.4	3.1 (1.9) ± 0.1	160
16	3.8	8.6	28.0 (18.3) ±11.5	46.3 (30.3) ± 0.6	1.7	3.0 (2.0) ± 0.2	105
17[m]	5.8	6.4	11.6 (11.2) ± 2.7	13.6 (13.1) ± 2.1	1.2	17.0 (16.4) ± 9.5	1468
18	2.2	8.6	38.5 (25.3) ± 1.1	57.4 (37.8) ± 2.1	1.5	3.1 (2.0) ± 0.1	81
19[m]	4.3	6.9	25.8 (24.9) ± 2.4	35.9 (34.7) ± 0.8	1.4	0.9 (0.6) ± 0.0	35
20	4.9	8.6 10.2	39.1 (27.9) ± 5.1	74.7 (53.3) ± 2.9	1.9	0.9 (1.0) ± 0.2	23
21[m]	7.0	10.2	31.8 (32.5) ± 5.7	33.1 (33.9) ± 2.7	1.0	> (100)	>3077
22[m]	6.8	6.9	5.5 (5.5) ± 0.4	10.6 (10.6) ± 1.2	1.9	> (100)	>18,182
23	4.8	8.5	27.2 (17.2) ± 3.3	59.1 (37.4) ± 4.3	2.2	16.0 (10.1) ± 3.2	588
24[m]	6.9	6.9	12.9 (12.2) ± 1.9	10.3 (9.7) ± 1.9	0.8	0.7 (0.6) ± 0.3	54
25	4.8	8.5	19.8 (13.1) ± 1.5	28.8 (19.1) ± 1.7	1.5	> (100)	>5051
26[m]	4.3	6.9	7.9 (7.4) ± 0.5	10.25 (9.6) ± 1.7	1.3	> (100)	>13,514
PM[f]	2.9	6.8	33,000 (8200) ± 3200	60,700 (15.1) ± 2900	1.8	271.0 (67.4) ± 23.7	8
DHA[g]	2.5		0.9 (0.26) ± 0.2	8.8 (2.5) ± 1.5	9.7	147.7 (42.0) ± 50.0	161,539
M[h]			0.71 ± 0.1 ng/mL	8.9 ± 2.2 ng/mL	12.4	31.4 ± 1.6 μg/mL	44,225
CQ[i]	4.69	7.5 10.4	13.8 (4.4) ± 4.7	300.0 (95.8) ± 2.8	22	nd[l]	–
AS[j]			<5.2	8.1 (3.1) ± 0.5		nd[l]	–
EM[k]						0.1 (0.1) ± 0.02	

[a] Minimum concentration of compound inducing 50% parasitic cells inhibition, data represents the mean of three independent experiments ± S.D. (standard deviation).
[b] Minimum concentration of compound selectively inducing 50% of mammalian CHO (Chinese Hamster Ovarian) cells.
[c] Calculated values using ACD Chem/Sketch [31].
[d] Resistance index (RI) = IC_{50} Dd2/IC_{50} NF54.
[e] Selectivity index (SI) = IC_{50} CHO/IC_{50} NF54.
[f] Pyrimethamine (PM).
[g] Dihydroartemisinin (DHA).
[h] Equimolar DHA:PM mixture (M).
[i] Chloroquine (CQ) was tested as diphosphate salt.
[j] Artesunate (AS).
[k] Emetine (EM) was used as the reference drug in the cytotoxicity study.
[l] Not determined (nd).
[m] Hybrid-dimer.

The ^{1}H NMR signals of the EDA linker protons were 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, due to integrating the four protons together. The COSY was 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 showed at δ 47. The HSQC spectra assisted in confirming the presence of these two signals. The piperidine moiety in compounds **14**, **16** and **17** had distinctive ^{1}H NMR signals at δ 3.72 and 1.71, arising from the alicyclic protons, while the alicyclic carbon atoms displayed signals at δ 44.29 and 26.20. The morpholine moiety in hybrids **15** and **18** and dimers **19** and **26** had ^{1}H NMR signals at δ 3.71–3.41, correlating with the ^{13}C NMR signals at δ 66.04 and 43.18 in the HSQC spectrum. The *p*-anisidine in hybrids and hybrid-dimers **16**–**22** and **25** showed 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 was visible at δ 55.45 in the ^{13}C NMR spectrum, whilst the aromatic carbon atoms resonated at δ 153.61, 129.89, 123.16 and 113.70. The aniline moiety of compounds **23**–**26** displayed 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 compounds **20** and **21** gave a characteristic ^{1}H NMR signal at δ 1.06 ppm, corresponding with the ^{13}C NMR signal at δ 20.24, while the signals resulting from the two methine groups gave an ^{1}H NMR signal at δ 2.68 and a ^{13}C NMR signal at δ 44.19.

Conversion of the secondary amine NH of the linker (EDA in the hybrids) to a tertiary amine in geminal dimers **17**, **19**, **21**, **22**, **24** and **26** resulted in the ^{13}C NMR signals of both C-b and C-1′ carbon atoms to shift from roughly δ 47 ppm–53 ppm, whilst the signal pertaining to C-2′ remained at δ 37 ppm. Similarly, the signals associated with H-1′ and H-b had shifted from δ 3.10 ppm–3.50 ppm, whilst H-2′ remained at the same position more or less, confirming that indeed the second coupling of compound **1** had occurred on the nitrogen amine group between C-b and C-1′ carbons to form the dimers.

3.2. Biological activity

All synthesized compounds were screened against the *P. falciparum* NF54 and Dd2 strains. The NF54 strain from African origin is susceptible to all known antimalarial drugs and is often the strain of choice for testing antigametocytocidal activity, presumably because of the highest gametocytemia level obtained with this strain in cell cultures [24]. The Dd2 strain, collected from Indochina/Laos, is chloroquine and mefloquine resistant and delivers a poor level of gametocytemia in cultures [24]. *Plasmodium* gametocytes, responsible for the malaria parasite transmission from humans to mosquitoes, represent an important target for new antimalarial drugs in an attempt to achieve malaria eradication. Gametocytes undergo complex development that is characterized by five morphologically distinct stages (I–V). The earliest developmental stages (I and II) of gametocytes are susceptible to most antimalarial drugs [25]. DHA, the active metabolite of artemisinins is highly active against stages I–III gametocytes, but has incomplete activity against stages IV and V gametocytes [25,26].

Cycloguanil, which contains a triazine pharmacophore, was unavailable for screening for comparison to the synthesized compounds. A pyrimidine derivative, *viz*. pyrimethamine, which shares the same biological mechanism of action (type 2 antifolates), was hence used as reference instead and screened alongside the synthesized compounds.

Artemisinin and triazine pharmacophores are known for their activity against malaria, either on their own, or in combination with others [10–14,27,28]. *In vivo*, most artemisinin derivatives are metabolized into DHA within a few minutes [28–30]. Arthemether and arteether, both ether derivatives of artemisinin, stay intact long enough to exercise their own antimalarial effects, before being metabolized into DHA, with all three drugs showing comparable potencies [28]. The synthesized hybrids and hybrid-dimers, also ethers, likewise exert their own antimalarial activities prior to metabolism into DHA.

The majority of the hybrids and hybrid-dimers either had secondary and/or tertiary amines, with calculated pK_a values, using ACD Chem/Sketch [31], varying in the 6–10 range. The pK_a values of each synthesized compound, as reported in Table 1, represented that of the nitrogen linking EDA to the artemisinin pharmacophore *via* the C-b carbon. In hybrids **14**, **15**, **16**, **18**, **20**, **23** and **25**, this nitrogen was basic and therefore had a pKa value of ~8. Such property was lost in dimers **17**, **19**, **21**, **22**, **24** and **26**, due to the attachment of the second artemisinin moiety, which resulted in a pKa value of ~6. Hybrid **20** and its dimer **21** further had the 2-(isopropylamino)ethylamino substituent on the triazine ring. The tertiary nitrogen of this group was very basic with a pK_a value around 10, which was comparable to the calculated pK_a value of 10.4 (side chain tertiary amine) of CQ. Compound **20** was the least active against the NF54 strain and the second to least active against the Dd2 strain. One of the suggested mechanisms of action of CQ is ion trapping, which relies on the protonation of weakly basic amino groups. Indeed, in the normal *in vivo* environment at pH 7.4, amines tend to be neutral and unprotonated, making it easy for them to cross lipophilic membranes. Upon reaching the acidic food vacuole of the malaria parasite, where the pH value is in the 4.5–5.5 range, these amines 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 [32,33]. This observation may thus imply that, unlike with CQ, ion trapping may not play a significant role in the activities of these amino groups containing compounds. The high activities of dimers **22** and **26**, despite having secondary amine groups (NH linked to C-b carbon), with low pK_a values in the 6–7 range, further supported such assumption.

Another plausible explanation for the low activity of hybrid **20** in relation to the high pK_a value (10.2) of its tertiary amine could be that this compound (unlike dimers **22** and **26**) was protonated at physiological pH 7.4. As it had therefore crossed the biomembranes' phospholipid layers with difficulty, it reached the parasites' digestive system in lesser concentrations.

It is known that in order for the uptake of a drug molecule to occur through a membrane, that drug must possess limiting aqueous solubility and be neither too lipophilic, nor too hydrophilic. Lipophilic drugs show poor aqueous solubility and tend to be taken up in fatty globules in the intestine. Once they reach the blood stream, they may be absorbed into tissue where their slow release exacerbates toxicity, such as neurotoxicity. Contrary, hydrophilic drugs may be excreted directly by the kidneys, or penetrate a cell membrane and become entrapped in intracellular aqueous media. An ideal drug must hence possess balanced lipophilic/hydrophilic properties in order to both permeate biological membranes and be taken up in the systemic circulation. The lipid/water partition coefficient (Log *P*) offers a good measure of this balance, with values between 1 and 5 being targeted, and 1–3 being ideal for better drug absorption through biological membranes [34,35]. Hybrid **18**, with calculated Log *P* value of 2.2, fitting nicely into the ideal range, happened to be one of the least active compounds during this study. On the contrary, hybrid-dimer **22**, with a Log *P* value (6.9) beyond that of both the targeted and ideal ranges, was found to be the most active against strain NF54 and the third most active against Dd2. These observations corroborated

earlier findings that properties, such as hydrogen bonding, molecular size and shape, polarity, flexibility and the charge/ionization of a compound/drug molecule as a whole (rather than a single property), affect its absorption through membranes [36].

Compounds **17**, **19**, **21** and **24** are the hybrid-dimers of monomeric hybrids **16**, **18**, **20** and **23**, respectively. Each dimer displayed slightly higher activity than its monomer counterpart against both the NF54 and Dd2 strains. However, this increase, which may presumably be as a result of more artemisinin reaching the site of action, appeared moderately more pronounced against the Dd2 than the NF54 strain.

Furthermore, the RI values of most hybrids and hybrid-dimers were in the 1–4 range, which may have been indicative of the CQR strain being on the verge of developing resistance against them. However, this strain was found to be more resistant towards both DHA (RI = 9) and CQ (RI = 22). As the main pharmacological shortcoming of most currently used antimalarial drugs is known as being their decreased activity against the resistant strains of *P. falciparum*, the favourable outcomes in this regard, as demonstrated by the compounds investigated during this study, possibly made them good candidates for further development and investigation as possible future antimalarials.

Most of the synthesized compounds, especially hybrid-dimers **22** and **26**, demonstrated a high level of selectivity towards the parasitic cells. They were also found to be the most antigametocytocidal of all hybrids and hybrid-dimers and had activities comparable to those of dihydroartemisinin, artesunate and the fixed DHA-PM (1:1) combination against the resistant CQ strain. These two dimers hence showed good potential as transmission blocking compounds, suitable for further screening/investigation. None of the hybrid and hybrid-dimers investigated during this study, however, displayed activities higher than AS or ArM against the NF54 strain, possibly suggesting that the triazine moiety had antagonized the antigametocytes action of artemisinin. A better partner drug would therefore be required in order to achieve antigametocytocidal synergism, using the artemisinin-based hybrid strategy. The choice of such a partner drug was further rendered necessary, due to the most active synthesized compounds in this study not demonstrating significant advantages over the equimolar combination of dihydroartemisinin–pyrimethamine.

4. Conclusion

During this study, a series of hybrids and hybrid-dimers were successfully synthesized by linking artemisinin and triazine pharmacophores through nucleophilic substitution, involving dihydroartemisinin and cyanuric chloride. Their chemical structures were confirmed through routine IR, NMR and HRMS techniques. The synthesized compounds were all active against the CQS and CQR Dd2 strains of *P. falciparum*, with good selectivity towards these parasitic cells in the presence of mammalian cells. Although all synthesized compounds were active against both strains, the dimers demonstrated higher activity than their monomeric counterparts. Dimers **22** and **26** were distinctively the most active and most selective, possessing potencies comparable to those of artesunate and dihydroartemisinin against the NF54 strain, while being slightly less active than both against the CQS strain. Hybrid-dimer **24** was the second most active against the Dd2 strain, but had lower selectivity index. The antimalarial activities of both compounds in conjunction with their low cytotoxicities may qualify them as potential drug candidates to be further investigated in search for novel, antigametocytocidal compounds for blocking malaria transmission from mosquitoes to humans. It would be interesting to establish whether their excellent *in vitro* nanomolar activities would also be apparent *in vivo*, and whether their activities were purely dependent on the artemisinin pharmacophore, or whether their folate biosynthetic pathways would also be implicated, or both. A dihydrofolate reductase (DHFR) assay would be required to ascertain their antifolate properties. These compounds also showed good potential as possible candidates for undergoing pharmacokinetic studies in order 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. Furthermore, a clear identification of the gametocyte stage of action may be useful in designing new compounds, using the hybrid strategy.

5. Materials and methods

5.1. Materials

Dihydroartemisinin (mixture of 10α and 10β epimers) was purchased from Changzhou Kaixuan Chemical Co (Chunjiang, China). Boron trifluoride diethyl etherate ($BF_3{\cdot}Et_2O$), 2-bromoethanol, *N*,*N*-dimethylformamide (DMF), morpholine, piperidine, aniline, cyanuric chloride (CyCl), tetrahydrofuran (THF), ethylenediamine (EDA), 2-(diisopropylamino)ethylamine and ammonia (NH_4OH) 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 were purchased from Unilab (Krugersdorp, South Africa). All chemicals, reagents and solvents, *viz.* ethyl acetate (EtOAc), petroleum ether (PE), dichloromethane (DCM) and methanol (MeOH) were of analytical grade and were used without further purification.

5.2. General chemical and analytical procedures

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

Mass spectra (MS) were recorded in positive mode by one of two mass spectrometers. The first was a Thermo Electron LXQ™ ion trap mass spectrometer, equipped with an APCI source set at 300 °C, with Xcalibur 2.2 data acquisition and analysis software, utilizing direct infusion with a Harvard syringe pump at a flow rate of 10 μL/min. A full scan from 100 to 1200 amu was generated in 1 s, at a capillary voltage of 7 V, while the corona discharge was set at 10 μA. The second instrument used was a Bruker MicroTOF Q II mass spectrometer, equipped with an APCI or an ESI source set at 300 °C or 180 °C, respectively, using Bruker Compass DataAnalysis 4.0 software. A full scan from 50 to 1500 *m*/*z* was generated at a capillary voltage of 4500 V, an end plate offset voltage of −500 V and a collision cell RF voltage of 100 V_{pp}.

Melting points (mp) were determined in duplicate on a BÜCHI melting point B-545 instrument and reported in degrees 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 equipment consisted of a continuously focused microwave power delivery

system with operator selectable power output from 0 to 300 W, a maximum current of 6.3 A and a frequency of 50/60 Hz. The temperature of the contents of the vessel was monitored using an IR sensor located underneath the reaction vessel. The contents of the vessel were 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.

5.3. Biological study

5.3.1. Determination of antimalarial activity

The synthesized compounds were screened against the NF54 and Dd2 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 [37]. Quantitative assessment of antiplasmodial activity *in vitro* was determined *via* the parasite lactate dehydrogenase (pLDH) assay, using a modified method, as described by Makler et al. [38]. The test samples were all tested in triplicate on one occasion.

The test samples were each prepared into 20 mg/mL stock solutions in 100% DMSO. These stock solutions were stored at −20 °C. Further dilutions were prepared in complete medium on the day of analysis. Samples were tested as a suspension if not completely dissolved. Chloroquine (CQ) and artesunate were used as reference drugs. A full dose response was performed to determine the concentration that would inhibit 50% of parasite growth (IC_{50} value). Test samples were tested at a starting concentration of 10 μg/mL and then serially diluted two-fold in complete medium to give a series of ten concentrations, with the lowest concentration being 0.02 μg/mL. The same dilution technique was used for all samples. References were tested at a starting concentration of 1000 ng/mL. The highest concentration of solvent to which the parasites were exposed to had no measurable effect on the parasite viability (data not shown).

5.3.2. Cytotoxicity assay

Test samples were screened for *in vitro* cytotoxicity against a mammalian cell line, Chinese Hamster Ovarian (CHO), using the 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazoliumbromide (MTT) assay. The MTT assay is used as a colorimetric test for cellular growth and survival and compares well with other available assays [39,40]. The tetrazolium salt of MTT was used to measure all growth and chemosensitivity of the mammalian cells. The samples were all tested in triplicate on one occasion.

The same stock solutions being prepared for antiplasmodial testing were used for cytotoxicity testing. Test compounds were stored at −20 °C until use. Dilutions were prepared on the day of analysis. Emetine was used as reference drug in all tests. The initial concentration of emetine was 100 μg/mL, which was serially diluted in complete medium with ten-fold dilutions to give six 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, integrating outcomes by using GraphPad Prism v.4 software.

5.4. Synthetic procedures

5.4.1. Monoamine substituted triazines **3**–**4**

Cyanuric chloride **2** (CyCl) was dissolved in acetone at 0 °C, after which Na_2CO_3 was added first and then the relevant amines in four portions (Scheme 1). The reaction mixture was allowed to stir for 6 h at 0 °C until completion, after which the solvent was evaporated *in vacuo*. The resulting solid residue was washed with ice water (3 × 50 mL), filtered and then purified through column chromatography, eluting with various ratios of solvents. In some instances the product was then further purified through recrystallization from DCM.

5.4.1.1. 4,6-Dichloro-N-(4-methoxyphenyl)-1,3,5-triazin-2-amine, ***3***. The reaction of **2** (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), followed by purification through silica gel column chromatography, eluting with EtOAc/PE (1:4, v/v) and recrystallization afforded **3** as a brown powder (8.0 g, 68%); mp: 170.9 °C; ^{1}H NMR (600 MHz, DMSO-d_6): 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-d_6) 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′); HRMS-APCI *m*/*z*: 271.0148 $[M + H]^+$ [$C_{10}H_9Cl_2N_4O$: 271.0175 calcd.].

5.4.1.2. 4,6-Dichloro-N-phenyl-1,3,5-triazin-2-amine, ***4***. The reaction of **2** (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), followed by purification through silica gel column chromatography, eluting with DCM afforded **4** as a white powder (4.5 g, 86%); mp: 199.5 °C; ^{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-d_6): 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′); HRMS-APCI *m*/*z*: 241.0028 $[M + H]^+$ [$C_9H_7Cl_2N_4$: 241.0070 calcd.].

5.4.2. Primary amino-functionalized triazines **5**–**13**

The synthesis occurred in two steps. First, to either **2** or **3** or **4**, dissolved in acetone at 0 °C, was added Na_2CO_3 and then the relevant amine in four portions. The reaction mixture was allowed to stir for 1–3 h at 0 °C and then for 3–24 h at room temperature, whereafter the solvent was evaporated *in vacuo*. The resulting residue was dissolved in either EtOAc or DCM and washed several times with water, then purified through column chromatography, eluting with various ratios of solvents. The resulting product was then used without further purification.

In the second step, the above synthesised di-substituted triazine intermediate was dissolved in EDA and heated with continuous stirring at 50–70 °C for 3–24 h. Afterwards, the reaction was quenched with ice cold water and the crude product extracted with EtOAc (3 × 100 mL), then purified through column chromatography, eluting with various ratios of solvents. The different steps are illustrated in Scheme 1.

5.4.2.1. N-(2-Aminoethyl)-4,6-bis(piperidin-1-yl)-1,3,5-triazin-2-amine, ***5***. The reaction of **2** (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), followed by purification through 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, 32 eq.), followed by chromatography with solvent mixtures MeOH:DCM:NH_4OH (1:13.5:0.5, v/v/v) and MeOH:NH_4OH (19:1, v/v) afforded **5** as a white powder (1.8 g, 83%); mp: 123.2 °C; ^{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′); HRMS-APCI *m*/*z*: 306.2390 $[M + H]^+$ [$C_{15}H_{28}N_7$: 306.2430 calcd.].

5.4.2.2. N-(2-Aminoethyl)-4,6-bis(morpholin-4-yl)-1,3,5-triazin-2-amine, ***6***. The reaction of **2** (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) and extraction with DCM, followed by purification through chromatography successively with EtOAc:DCM (1:1, v/v) and 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 eq.), followed by extraction with EtOAc and then purification through chromatography, using MeOH:DCM:NH_4OH (1:4:0.5, then 1:9:0.5, v/v/v) afforded **7** as a white powder (1 g, 49%); mp: 133.2 °C; IR (ATR)/cm^{-1}: 3369, 3263, 3004, 2933, 2850; ^{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′); HRMS-APCI m/z: 310.1972 $[M + H]^+$ $[C_{13}H_{24}N_7O_2$: 310.2013 calcd.].

*5.4.2.3. 2N-(2-Aminoethyl)-4N-(4-methoxyphenyl)-6-(piperidin-1-yl)-1,3,5-triazine-2,4-diamine, **7**.* The reaction of **3** (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), followed by extraction with EtOAc, then purification through chromatography, eluting with EtOAc:PE (1:4, v/v) 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 eq.), followed by extraction with EtOAc and purification through chromatography, eluting with MeOH:DCM:NH_4OH (1:14:0.5, v/v/v) resulted in compound **6** as a white powder (3.5 g, 58%); mp: 132.8 °C; IR (ATR)/cm^{-1}: 3345, 3279, 2966, 2896, 2858; ^{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′); HRMS-APCI m/z: 344.2175 $[M + H]^+$ $[C_{17}H_{26}N_7O$: 344.2221 calcd.].

*5.4.2.4. 2-N-(2-Aminoethyl)-4-N-(4-methoxyphenyl)-6-(morpholin-4-yl)-1,3,5-triazine-2,4-diamine, **8**.* The reaction of **3** (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) and extraction with EtOAc, followed by purification through chromatography with EtOAc:PE (1:1, v/v) 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 eq.) and extraction with EtOAc, followed by purification through chromatography with MeOH:DCM:NH_4OH (1:9:0.5, v/v/v) afforded **8** as a white powder (1.8 g, 85%); mp: 154.6 °C; IR (ATR)/cm^{-1}: 3397, 3265, 2927, 2864, 1574; ^{1}H NMR (600 MHz, DMSO-d_6): 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-d_6): 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′); HRMS-APCI m/z: 346.1965 $[M + H]^+$ $[C_{16}H_{24}N_7O_2$: 346.2013 calcd.].

*5.4.2.5. 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, **9**.* Compound **3** (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) afforded 2.5 g of the intermediate as a brown powder after extraction with EtOAc, followed by purification through chromatography, using MeOH:DCM (7:3, v/v) as eluent.

The reaction of the intermediate (5.2 mmol, 2 g) with EDA (448.3 mmol, 30 mL, 86 eq.) and extraction with EtOAc, followed by purification through chromatography with MeOH:DCM:NH_4OH (1:4:0.5, v/v/v) resulted in **9** as a white powder (2.1 g, 99%); mp: 68.3 °C; IR (ATR)/cm^{-1}: 3391, 3271, 2930, 2859; ^{1}H 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′); HRMS-APCI m/z: 403.2908 $[M + H]^+$ $[C_{20}H_{35}N_8O$: 403.2956 calcd.].

*5.4.2.6. 2-N-(2-Aminoethyl)-4-N,6-N-bis(4-methoxyphenyl)-1,3,5-triazine-2,4,6-triamine, **10**.* The reaction of **2** (27.1 mmol, 5 g), Na_2CO_3 (54.2 mmol, 15.5 g, 2 eq.) and *p*-anisidine (54.2 mmol, 6.6 g, 2 eq.) in acetone (80 mL), followed by extraction with EtOAc, then purification through chromatography, eluting with EtOAc:PE (1:4, v/v) afforded 4.2 g of the intermediate as a brown powder.

The reaction of the intermediate (11.8 mmol, 4.2 g) with EDA (373.5 mmol, 25 mL, 32 eq.) and extraction with EtOAc, followed by purification through chromatography with MeOH:DCM:NH_4OH (1:4:0.2, v/v/v) afforded 3.2 g (71%) of **10** as a white powder; mp: 150.1 °C; IR (ATR)/cm^{-1}: 3267, 2964, 1602, 1571; ^{1}H NMR (600 MHz, DMSO-d_6): δ 7.64 (s, 4H, H-6′), 6.82 (d, J = 7.8 Hz, 5H, H-7′), 3.70 (s, 6H, H-9′), 3.29–3.26 (m, 2H, H-2′), 2.68 (t, J = 6.2 Hz, 2H, H-1′); ^{13}C NMR (151 MHz, DMSO-d_6): δ 165.89 (C-3′), 163.88 (C-4′), 154.29 (C-8′), 133.70 (C-5′), 121.41 (C-6′), 113.42 (C-7′), 55.05 (C-9′), 43.86 (C-2′), 41.45 (C-1′); HRMS-APCI m/z: 382.1959 $[M + H]^+$ $[C_{19}H_{23}N_7O_2$: 382.1923 calcd.].

*5.4.2.7. 2-N-(2-Aminoethyl)-4-N,6-N-diphenyl-1,3,5-triazine-2,4,6-triamine, **11**.* The reaction of **2** (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) and extraction with EtOAc, followed by purification through chromatography 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, 28 eq.) and extraction with EtOAc, followed by purification through chromatography with MeOH:DCM:NH_4OH (1:4:0.2, v/v/v) afforded **11** as a white powder (2.9 g, 67%); mp: 77.7 °C; IR (ATR)/cm^{-1}: 3366, 3280, 3168, 2935, 2835; ^{1}H NMR (600 MHz, DMSO-d_6): 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-d_6): 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′); HRMS-APCI m/z: 322.1754 $[M + H]^+$ $[C_{17}H_{20}N_7$: 322.1780 calcd.].

*5.4.2.8. 2-N-(2-Aminoethyl)-4-N-(4-methoxyphenyl)-6-N-phenyl-1,3,5-triazine-2,4,6-triamine, **12**.* The reaction of **4** (8.3 mmol, 2 g), Na_2CO_3 (16.6 mmol, 4.7 g, 2 eq.) and p-anisidine (8.3 mmol, 1.0 g, 1 eq.) in acetone (40 mL), followed by extraction with EtOAc, then purification through chromatography with EtOAc:PE (1:4, v/v) afforded 2.3 g of the intermediate as off-white powder.

The reaction of the intermediate (6.1 mmol, 2 g) with EDA (448.3 mmol, 30 mL, 7 eq.) and extraction with EtOAc, followed by purification through chromatography with MeOH:DCM:NH_4OH (1:4:0.5, v/v/v) afforded **12** as a purple powder (1.8 g, 84%); mp: 134.5 °C; IR (ATR)/cm^{-1}: 3271, 2961, 2930, 2870, 2834; ^{1}H NMR (600 MHz, DMSO-d_6) 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-d_6): 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′); HRMS-APCI m/z: 352.1864 $[M + H]^+$ $[C_{18}H_{22}N_7O$: 352.1908 calcd.].

*5.4.2.9. 2-N-(2-Aminoethyl)-6-(morpholin-4-yl)-4-N-phenyl-1,3,5-triazine-2,4-diamine, **13**.* The reaction of **4** (16.6 mmol, 4 g), Na_2CO_3 (33.2 mmol, 9.4 g, 2 eq.) and morpholine (16.6 mmol, 1.5 mL, 1 eq.) in acetone (80 mL), followed by extraction with EtOAc, then purification through chromatography, eluting with EtOAc:PE (1:4, v/v) afforded 2.2 g of the intermediate as a white powder.

The reaction of the intermediate (6.9 mmol, 2 g) with EDA (373.5 mmol, 25 mL, 54 eq.), followed by extraction with EtOAc, then purification through chromatography, eluting with MeOH:DCM:NH_4OH (1:9:0.5, v/v/v) afforded compound **13** as a white powder (1.8 g, 85%); mp: 95.6 °C; IR (ATR)/cm^{-1}: 3270, 2953, 2923, 2854; ^{1}H NMR (600 MHz, $CDCl_3$): δ 7.52 (s, 2H, H-9′), 7.26 (dd, J = 13.5, 5.9 Hz, 2H, H-10′), 6.98 (t, J = 7.3 Hz, 1H, H-11′), 3.73 (d, J = 43.1 Hz, 8H, H-6′, H-7′), 3.44 (q, J = 5.9 Hz, 2H, H-2′), 2.87 (t, J = 5.8 Hz, 2H, H-1′); ^{13}C NMR (151 MHz, $CDCl_3$): δ 166.28 (C-3′), 165.40 (C-5′), 164.26 (C-4′), 139.45 (C-8′), 128.51 (C-10′), 122.29 (C-11′), 120.00 (C-9′), 66.58 (C-7′), 43.62(C-6′), 43.11 (C-2′), 41.34 (C-1′); HRMS-APCI m/z: 316.1863 $[M + H]^+$ [$C_{15}H_{21}N_7O$: 316.1818 calcd.].

*5.4.3. Artemisinin–triazine hybrids and hybrid-dimers **14–26***

DHA derivative **1** and triazine intermediates **5–13**, 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 1). This sequence was repeated until the reaction was complete. The solvent was then evaporated *in vacuo*, the resulting residue dissolved in EtOAc and then washed with H_2O. The organic phase was dried over $MgSO_4$ and concentrated *in vacuo*, resulting in an oil. The purification through silica gel column chromatography, eluting with various ratios of MeOH, DCM, EtOAc, PE and NH_4OH, afforded the target DHA monomeric- and/or dimeric (in some cases) hybrid free base compounds, which were later converted into oxalate salts.

*5.4.3.1. (2-{[Bis(piperidin-1-yl)-1,3,5-triazin-2-yl]amino}ethyl)-2-(10β-dihydroartemisinoxy)ethylamine (oxalate) **14**.* The reaction of **1** (2.9 mmol, 1.1 g), K_2CO_3 (5.8 mmol, 0.8 g, 2 eq.) and **5** (2.9 mmol, 0.9 g, 1 eq.) in DMF (25 mL), followed by purification through chromatography successively with MeOH:DCM (1:4, v/v) and MeOH, after conversion afforded **14** salt as a white powder (0.4 g, 20%); $C_{32}H_{53}N_7O_5$; mp: 152.2 °C; IR (ATR)/cm^{-1}: 3407, 2933, 2551, 1721; ^{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 (dt, 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 (dt, 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 (C-oxalate, 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); HRMS-APCI m/z: 616.4182 $[M + H]^+$ [$C_{32}H_{54}N_7O_5$: 616.4208 calcd.].

*5.4.3.2. (2-{[Bis(morpholin-4-yl)-1,3,5-triazin-2-yl]amino}ethyl)-2-(10β-dihydroartemisinoxy)ethylamine (oxalate) **15**.* The reaction of **1** (1.6 mmol, 0.6 g), K_2CO_3 (3.2 mmol, 0.5 g, 2 eq.) and **6** (1.6 mmol, 0.5 g, 1 eq.) in DMF (25 mL), followed by purification through chromatography with MeOH:DCM (1:9, v/v), afforded after conversion, **15** as a white oxalate salt powder (0.3 g, 28%); mp: 162.1 °C; $C_{30}H_{49}N_7O_7$; IR (ATR)/cm^{-1}: 3400, 2920, 2848; ^{1}H NMR (600 MHz, DMSO-d_6): 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-d_6): δ 165.72 (C-Oxalate, 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); HRMS-APCI m/z: 620.3758 $[M + H]^+$ [$C_{30}H_{50}N_7O_7$: 620.3794 calcd.].

*5.4.3.3. 2-N-(4-Methoxyphenyl)-6-(piperidin-1-yl)-4-N-{2-[(2-(10β-dihydroartemisinoxy) ethyl)amino]ethyl}-1,3,5-triazine-2,4-diamine (oxalate) **16**.* The reaction of **1** (5.8 mmol, 2.3 g), K_2CO_3 (11.6 mmol, 1.6 g, 2 eq.) and **7** (5.8 mmol, 2 g, 1 eq.) in DMF (50 mL), followed by purification through chromatography with MeOH:EtOAc:NH_4OH (1:14:0.5 and 19:1:0.5, v/v/v), after conversion resulted in **16** salt as a white powder (0.5 g, 12%); mp: 151.5 °C; $C_{34}H_{51}N_7O_6$; IR (ATR)/cm^{-1}: 3266, 2935, 2860, 1719; ^{1}H NMR (600 MHz, $CDCl_3$): δ 7.45 (d, J = 8.2 Hz, 2H, H-7′), 6.75 (d, J = 8.5 Hz, 2H, H-8′), 5.40–5.26 (m, 1H, H-12), 4.75 (d, J = 3.4 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-11′), 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-13′, H-12′, 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 (C-Oxalate), 153.61 (C-9′), 129.89 (C-6′), 123.16 (C-7′), 113.70 (C-8′), 103.93 (C-3), 102.10 (C-10), 87.98 (C-12), 81.03 (C-12a), 63.89 (C-a), 55.45 (C-10′), 52.44 (C-5a), 47.58 (C-1′, C-b), 45.19 (C-11′), 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-12′), 24.29(C-5, C-13′), 24.14 (C-8), 20.25 (C-15), 12.89 (C-16); HRMS-APCI m/z: 654.3970 $[M + H]^+$ [$C_{34}H_{52}N_7O_6$: 654.4001 calcd.].

*5.4.3.4. 2-N-{2-[Bis(2-{10β-dihydroartemisinoxy}ethyl)amino]ethyl}-4-N-(4-methoxyphenyl)-6-(piperidin-1-yl)-1,3,5-triazine-2,4-diamine (oxalate) **17**.* The preceding reaction also yielded a dimeric compound, which after conversion afforded **17** salt as a white powder (0.3 g, 5%); mp: 139.4 °C; $C_{51}H_{77}N_7O_{11}$; IR (ATR)/cm^{-1}: 3262, 2939, 2874, 1717; ^{1}H NMR (600 MHz, $CDCl_3$): δ 7.45 (t, J = 18.7 Hz, 2H, H-7′), 6.79 (t, J = 22.0 Hz, 2H, H-8′), 5.48–5.26 (m, 2H, H-12), 4.78 (d, J = 3.1 Hz, 2H, H-10), 4.26 (dd, J = 29.4, 22.5 Hz, 2H, H-aα), 4.04–3.41 (m, 17H, H-aβ, H-2′, H-11′, H-10′, H-1′, H-b), 2.70–2.56 (m, 2H, H-9), 2.31 (td, J = 14.2, 3.7 Hz, 2H, H-4α), 2.06–1.94 (m, 2H, H-4β), 1.90–1.76 (m, 2H, H-5α), 1.76–1.52 (m, 12H, H-8α, H-8β, H-7β, H-12′, H-13′), 1.48–1.11 (m, 14H, H-6, H-5β, H-8a, H-14, H-5a), 0.98–0.70 (m, 13H, H-7α, H-15, H-16); ^{13}C NMR (151 MHz, $CDCl_3$): δ 163.22 (C-Oxalate), 161.00 (C-5′), 156.51 (C-3′), 155.63 (C-4′), 153.37 (C-9′), 129.65 (C-6′), 122.98 (C-7′), 113.78 (C-8′), 104.33 (C-3), 102.17 (C-10), 87.89 (C-12), 80.69 (C-12a), 62.71 (C-a), 55.26 (C-10′) 53.01 (C-1′, C-b), 52.37 (C-5a), 45.55 (C-11′), 43.99 (C-8a), 37.44 (C-6), 36.18 (C-4), 35.91 (C-2′), 34.26 (C-7), 30.47 (C-9), 26.01 (C-14), 25.50 (C-12′), 24.38 (C-5, C-8), 23.91 (C-13′), 20.29 (C-15), 12.77 (C-16); HRMS-APCI m/z: 964.5755 $[M + H]^+$ [$C_{51}H_{77}N_7O_{11}$: 964.5691 calcd.].

*5.4.3.5. 2-N-(4-Methoxyphenyl)-6-(morpholin-4-yl)-4-N-{2-[(2-{10β dihydroartemisinoxy}ethyl)amino]ethyl}-1,3,5-triazine-2,4-diamine (oxalate) **18**.* The reaction of **1** (2.9 mmol, 1.1 g), K_2CO_3 (5.8 mmol, 0.8 g, 2 eq.) and **8** (2.9 mmol, 1 g, 1 eq.) in DMF (50 mL), followed by chromatography successively with MeOH:EtOAc:PE:NH_4OH (1:10:4:0.5, v/v/v/v) and MeOH:EtOAc:NH_4OH (1:19:0.5, v/v/v) after conversion afforded **18** salt as a white powder

(0.3 g, 13%); mp: 140.9 °C; $C_{33}H_{49}N_7O_7$; IR (ATR)/cm^{-1}: 3268, 2919, 2861, 1719; ^1H 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 = 3.2 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 (Oxalate), 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); HRMS-APCI m/z: 656.3752 $[M + H]^+$ [$C_{33}H_{50}N_7O_7$: 656.3797 calcd.].

5.4.3.6. 2-N-{2-[Bis(2-{10β-dihydroartemisinoxy}ethyl)amino] ethyl}-4-N-(4-methoxyphenyl)-6-(morpholin-4-yl)-1,3,5-triazine-2,4-diamine (oxalate) ***19****.* The preceding reaction also yielded a dimeric compound, which after conversion afforded **19** salt as a white powder (0.1 g, 4%); mp: 145.3 °C; $C_{50}H_{75}N_7O_{12}$; IR (ATR)/cm^{-1}: 3248, 2940, 2920, 2874, 1719; ^1H NMR (600 MHz, $CDCl_3$): δ 7.41 (t, J = 13.1 Hz, 2H, H-7′), 6.81 (dd, J = 37.7, 7.1 Hz, 2H, H-8′), 5.43–5.30 (m, 2H, H-12), 4.84–4.69 (d, J = 3.5, 2H, H-10), 4.32–4.16 (m, 2H, H-aα), 4.04–3.31 (m, 22H, H-aβ, H-b, H-1′, H-2′, H-10′, H-11′, H-12′), 2.70–2.54 (m, 2H, H-9), 2.31 (td, J = 14.2, 3.4 Hz, 2H, H-4α), 2.00 (t, J = 23.8 Hz, 2H, H-4β), 1.90–1.78 (m, 2H, H-5α), 1.61 (dt, J = 26.8, 12.1 Hz, 6H, H-8α, H-8β, H-7β), 1.48–1.13 (m, 16H, H-5a, H-6, H-8a, H-5β, H-14), 0.98–0.74 (m, 15H, H-7α, H-15, H-16); ^{13}C NMR (151 MHz, $CDCl_3$): δ 162.88 (C-Oxalate), 161.65 (C-4′), 156.88 (C-3′), 155.75 (C-5′), 153.21 (C-9′), 129.20 (C-6′), 123.29 (C-7′), 113.93 (C-8′), 104.39 (C-3), 102.33 (C-10), 87.82 (C-12), 80.69 (C-12a), 66.35 (C-12′), 62.54 (C-a), 55.40 (C-10′), 53.17 (C-1′), 52.87 (C-b), 52.35 (C-5a), 44.59 (C-11′), 43.98 (C-8a), 37.29 (C-6), 36.17 (C-4), 35.83 (C-2′), 34.40 (C-7), 30.46 (C-9), 25.85 (C-14), 24.56 (C-5), 24.38 (C-8), 20.30 (C-15), 12.77 (C-16); HRMS-APCI m/z: 966.5553 $[M + H]^+$ [$C_{50}H_{75}N_7O_{12}$: 966.5483 calcd.].

5.4.3.7. 2-N-{2-[Bis(propan-2-yl)amino]ethyl}-4-N-(4-methoxyphenyl)-6-N-{2-[(2-{10β-dihydroartemisinoxy}ethyl)amino] ethyl}-1,3,5-triazine-2,4,6-triamine (oxalate) ***20****.* The reaction of **1** (5.0 mmol, 1.9 g), K_2CO_3 (9.9 mmol, 1.4 g, 2 eq.) and **9** (5.0 mmol, 2.0 g, 1 eq.) in DMF (50 mL), followed by chromatography successively with MeOH:DCM:NH_4OH (1:9:0.5, v/v/v) and MeOH:EtOAc:NH_4OH (1:14:0.5, v/v/v) after conversion afforded **20** salt as a white powder (0.5 g, 11%); mp: 152.0 °C; $C_{37}H_{60}N_8O_6$; IR (ATR)/cm^{-1}: 3255, 2937, 2877, 1718; ^1H 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 (d, J = 3.1 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); HRMS-APCI m/z: 713.469 $[M + H]^+$ [$C_{37}H_{61}N_8O_6$: 713.444 calcd.].

5.4.3.8. 2-N-{2-[Bis(2-{10β-dihydroartemisinoxy}ethyl)amino] ethyl}-4-N-{2-[bis(propan-2-yl)amino]ethyl}-6-N-(4-methoxyphenyl)-1,3,5-triazine-2,4,6-triamin (oxalate) ***21****.* The preceding reaction also gave a hybrid-dimer, which after conversion resulted in **21** salt as a white powder (0.4 g, 7%); mp: 141.9 °C; $C_{54}H_{86}N_8O_{11}$; IR (ATR)/cm^{-1}: 3254, 2947, 2875, 1719; ^1H NMR (600 MHz, DMSO-d_6): δ 7.61 (d, J = 19.3 Hz, 2H, H-7′), 6.76 (d, J = 8.9 Hz, 2H, H-8′), 5.33–5.25 (m, 2H, H-12), 4.65 (s, 2H, H-10), 3.78–3.71 (m, 2H, H-aα), 3.68 (s, 3H, H-10′), 2.97 (dd, J = 12.7, 6.3 Hz, 2H, H-13′), 2.75–2.64 (m, 6H, H-b, H-1′), 2.37 (s, 2H, H-9), 2.18–2.09 (m, 2H, H-4α), 1.93 (t, J = 20.8 Hz, 2H, H-4β), 1.73 (dd, J = 25.1, 12.7 Hz, 4H, H-5α, H-8α), 1.63–1.44 (m, 4H, H-8β, H-7β), 1.36–1.22 (m, 12H, H-6, H-8a, H-5β, H-14), 1.13–1.06 (m, 2H, H-5a), 0.96 (s, 12H, H-14′), 0.82 (d, J = 7.1 Hz, 14H, H-7α, H-15, H-16); ^{13}C NMR (151 MHz, DMSO-d_6): δ 153.95 (C-5′), 133.97 (C-6′), 121.06 (C-7′), 113.39 (C-8′), 103.26 (C-3), 101.20 (C-10), 86.91 (C-12), 80.50 C-12a), 66.57 (C-a), 55.10 (C-10′), 53.99 (C-b), 53.63 (C-1′), 52.06 (C-5a), 48.54 (C-12′), 48.10 (C-13′), 43.80 (C-8a), 40.13(C-2′), 39.78 (C-11′), 36.78 (C-6), 36.18 (C-4), 34.11 (C-7), 30.47 (C-9), 25.50 (C-14), 24.26 (C-5), 23.95 (C-8), 20.57 (C-15), 20.09 (C-14′), 12.82 (C-16); HRMS-APCI m/z: 1023.6457 $[M + H]^+$ [$C_{54}H_{86}N_8O_{11}$: 1023.6426 calcd.].

5.4.3.9. 2-N-{2-[Bis(2-{10β-dihydroartemisinoxy}ethyl)amino] ethyl}-4-N,6-N-bis(4-methoxyphenyl)-1,3,5-triazine-2,4,6-triamine (oxalate) ***22****.* The reaction of **1** (2.6 mmol, 1 g) and **10** (2.6 mmol, 1.0 g, 1 eq.) with K_2CO_3 (5.24 mmol, 0.7 g, 2 eq.) in DMF (25 mL), followed by chromatography successively with EtOAc and MeOH:DCM (1:14, v/v) after conversion afforded hybrid-dimer **22** salt as a white powder (0.1 g, 3%); mp: 147.4 °C; $C_{53}H_{75}N_7O_{12}$; IR (ATR)/cm^{-1}: 3269, 2944, 2875, 1718; ^1H NMR (600 MHz, DMSO-d_6): δ 7.62 (s, 4H, H-6′), 6.83 (s, 5H, H-7′), 5.34 (s, 2H, H-12), 4.70 (s, 2H, H-10), 3.88 (d, J = 11.9 Hz, 3H, H-aα), 3.79–3.40 (m, 11H, H–H-aβ, H-9′, H-2′), 3.16 (d, J = 15.0 Hz, 6H, H-1′, H-b), 2.39 (s, 2H, H-9), 2.16 (t, J = 12.7 Hz, 2H, H-4α), 2.05–1.89 (m, 2H, H-4β), 1.85–1.53 (m, 6H, H-8α, H-5α, H-8β), 1.47 (d, J = 10.8 Hz, 2H, H-7β), 1.40–1.16 (m, 12H, H-6, H-5β, H-8a, H-14), 1.11 (dd, J = 17.4, 10.8 Hz, 2H, H-5a), 0.83 (dd, J = 11.0, 6.6 Hz, 15H, H-7α, H-15, H-16); ^{13}C NMR (151 MHz, DMSO-d_6): δ 165.59 (C-3′), 163.77 (C-4′), 162.69 (C-oxalate), 154.40 (C-8′), 133.31 (C-5′), 121.78 (C-6′), 113.47 (C-7′), 103.44 (C-3), 101.02 (C-10), 86.90 (C-12), 80.46 (C-12a), 55.16 (C-9′), 53.15 (C-1′, C-b), 52.08 (C-5a), 43.57 (C-8a), 36.63 (C-6, C-2′), 36.02 (C-4), 33.70 (C-7), 30.44 (C-9), 25.48 (C-14), 24.12 (C-5), 23.69 (C-8), 20.12 (C-15), 12.55 (C-16); HRMS-APCI m/z: 1002.5512 $[M + H]^+$ [$C_{53}H_{75}N_7O_{12}$: 1002.5484 calcd.].

5.4.3.10. 2-N,4-N-Diphenyl-6-N-{2-[(2-{10β-dihydroartemisinoxy} ethyl)amino]ethyl} 1,3,5-triazine-2,4,6-triamine (oxalate) ***23****.* The reaction of **1** (2.3 mmol, 0.9 g), K_2CO_3 (4.6 mmol, 0.6 g, 2 eq.) and **11** (2.3 mmol, 0.7 g, 1 eq.) in DMF (25 mL), followed by chromatography with MeOH:EtOAc:NH_4OH (1:29:0.5 and 1:9:0.5, v/v/v) after conversion afforded **23** salt as a white powder (0.2 g, 14%); mp: 136.8 °C; $C_{34}H_{45}N_7O_5$; IR (ATR)/cm^{-1}: 3270, 2922, 2874, 1710; ^1H NMR (600 MHz, DMSO-d_6): 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), 0.91–0.59 (m, 8H, H-7α, H-15, H-16); ^{13}C NMR (151 MHz, DMSO-d_6): δ 165.73 (C-3′), 163.89 (C-4′), 163.06 (C-oxalate), 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); HRMS-APCI *m/z*: 632.3540 $[M + H]^+$ [$C_{34}H_{46}N_7O_5$: 632.3582 calcd.].

5.4.3.11. 2-N-{2-[Bis(2-{10β-dihydroartemisinoxy}ethyl)amino] ethyl}-4-N,6-N-diphenyl-1,3,5-triazine-2,4,6-triamine (oxalate) ***24***. The preceding reaction also gave hybrid-dimer **24**, which after conversion resulted in an off-white powder salt (0.1 g, 2.5%); mp: 109.3 °C; $C_{51}H_{71}N_7O_{10}$; IR (ATR)/cm^{-1}: 3290, 2941, 2876; ^{1}H NMR (600 MHz, $CDCl_3$): δ 7.56 (d, *J* = 24.3 Hz, 4H, H-6′), 7.29 (t, *J* = 7.9 Hz, 4H, H-7′), 7.05 (q, *J* = 7.1 Hz, 2H, H-8′), 5.37 (d, *J* = 16.7 Hz, 2H, H-12), 4.76 (d, *J* = 5.6 Hz, 2H, H-10), 3.98 (d, *J* = 4.7 Hz, 2H, H-aα), 3.54 (dd, *J* = 57.1, 21.8 Hz, 4H, H-aβ, H-2′), 2.99 (d, *J* = 124.6 Hz, 6H, H-b, H-1′), 2.64–2.56 (m, 2H, H-9), 2.31 (dt, *J* = 15.0, 10.8 Hz, 2H, H-4α), 2.05–1.96 (m, 4H, H-4β, H-5α), 1.85–1.81 (m, 2H, H-8α), 1.71–1.67 (m, 2H, H-8β), 1.56 (dd, *J* = 13.0, 2.8 Hz, 2H, H-7β), 1.44–1.35 (m, 10H, H-8a, H-5β, H-14), 1.30–1.17 (m, 4H, H-6, H-5a), 0.93–0.82 (m, 14H, H-7α, H-15, H-16); ^{13}C NMR (151 MHz, $CDCl_3$): δ 167.66 (C-3′, C-4′), 128.69 (C-7′), 123.44 (C-8′), 120.59 (C-6′), 104.34 (C-3), 102.17 (C-10), 87.93 (C-12), 80.34 (C-12a), 68.28 (C-a), 54.01 (C-1′), 53.83 (C-b), 52.39 (C-5a), 43.97 (C-8a), 37.32 (C-2′), 36.64 (C-4), 36.36 (C-6), 34.52 (C-7), 30.60 (C-9), 25.85 (C-14), 24.88 (C-5), 24.37 (C-8), 20.32 (C-15), 12.94 (C-16); HRMS-APCI *m/z*: 942.5322 $[M + H]^+$ [$C_{51}H_{71}N_7O_{10}$: 942.5262 calcd.].

5.4.3.12. 2-N-(4-Methoxyphenyl)-4-N-phenyl-6-N-{2-[(2-{10β-dihydroartemisinoxy}ethyl)amino]ethyl}-1,3,5-triazine-2,4,6-triamine oxalate ***25***. The reaction of **1** (5.7 mmol, 2.2 g), K_2CO_3 (11.4 mmol, 1.6 g, 2 eq.) and **12** (5.7 mmol, 2 g, 1 eq.) in DMF (50 mL), followed by chromatography with MeOH:DCM (1:4, v/v) and MeOH:DCM:NH_4OH (1:9:0.5, v/v/v), after conversion afforded **25** salt as a white powder (0.6 g, 14%); mp: 156.0 °C; $C_{35}H_{47}N_7O_6$; IR (ATR)/cm^{-1}: 3259, 2924, 2873, 1710; ^{1}H NMR (600 MHz, DMSO-d_6): 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* = 3.4 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, DMSO-d_6): 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); HRMS-APCI *m/z*: 662.3656 $[M + H]^+$ [$C_{35}H_{48}N_7O_6$: 662.3688 calcd.].

5.4.3.13. 2-N-{2-[Bis(2-{10β-dihydroartemisinoxy}ethyl)amino] ethyl}-6-(morpholin-4-yl)-4-N-phenyl-1,3,5-triazine-2,4-diamine (oxalate) ***26***. The reaction of **1** (3.2 mmol, 1.2 g) and **13** (3.2 mmol, 1.0 g, 1 eq.) with K_2CO_3 (6.3 mmol, 0.9 g, 2 eq.) in DMF (40 mL), followed by chromatography successively with MeOH:EtOAc:NH_4OH (1:14:0.5, v/v/v) and EtOAc:PE:NH_4OH (39:1:0.5, v/v/v) afforded hybrid-dimer **26**, which after conversion resulted in a white salt powder (0.3 g, 11%); mp: 143.7 °C; $C_{49}H_{73}N_7O_{11}$; IR (ATR)/cm^{-1}: 3273, 2940, 2914, 2875, 1730; ^{1}H NMR (600 MHz, $CDCl_3$) δ 7.48 (d, *J* = 7.9 Hz, 2H, H-9′), 7.20–7.08 (m, 2H, H-10′), 7.01 (t, *J* = 7.4 Hz, 1H, H-11′), 5.36 (d, *J* = 40.0 Hz, 2H, H-12), 4.76 (d, *J* = 3.4 Hz, 2H, H-10), 4.23–4.10 (m, 2H, H-aα), 3.99–3.62 (m, 13H, H-aβ, H-6′, H-2′,H-7′), 3.47 (td, *J* = 24.4, 20.7, 17.9 Hz, 6H, H-1′, H-b), 2.73–2.54 (m, 2H, H-9), 2.32 (dt, *J* = 14.2, 3.8 Hz, 2H, H-4α), 2.00 (t, *J* = 16.0 Hz, 2H, H-4β), 1.90–1.77 (m, 2H, H-5α), 1.61 (td, *J* = 31.2, 26.6, 12.1 Hz, 6H, H-8α, H-7β, H-8β), 1.49–1.26 (m, 12H, H-6, H-5β, H-8a, H-14), 1.25–1.11 (m, 3H, H-5a), 0.99–0.70 (m, 14H, H-7α, H-15, H-16); ^{13}C NMR (151 MHz, $CDCl_3$) δ 164.50 (C-oxalate), 164.07 (C-3′, C-5), 161.86 (C-4′), 136.83 (C-8′), 128.53 (C-10′), 124.55 (C-11′), 121.59 (C-9′), 104.14 (C-3), 102.36 (C-10), 87.86 (C-12), 80.91 (C-12a), 66.38 (C-7′), 63.02 (C-a), 53.45(C-1′), 52.30 (C-5a, C-b), 44.52 (C-6′), 43.86 (C-8a), 37.16 (C-6), 36.21 (C-2′,C-4), 34.22 (C-7), 30.42 (C-9), 25.98 (C-14), 24.41 (C-5, C-8), 20.41 (C-15), 13.00 (C-16); HRMS-APCI *m/z*: 936.5439 $[M + H]^+$ [$C_{49}H_{73}N_7O_{11}$: 936.5378 calcd.].

Conflict of interest

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

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Appendix A. Supplementary data

Supplementary data related to this article can be found at http://dx.doi.org/10.1016/j.ejmech.2014.01.040.

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