Chapter 6

Synthesis, antimalarial activity and cytotoxicity of dimeric artemisinin triazine hybrids – Article 4

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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]*

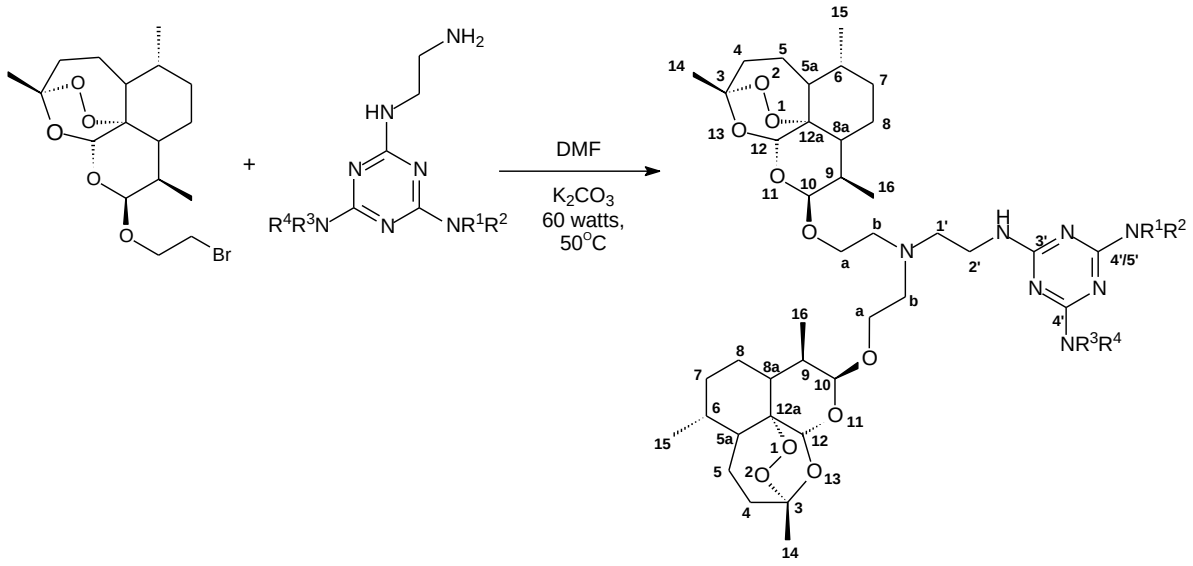
[a] Department of Pharmaceutical Chemistry, North-West University, Potchefstroom 2520, South Africa

[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



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[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

Abstract

In this study six dimeric artemisinin triazine hybrids were synthesised and their antimalarial activity against both the chloroquine sensitive (3D7) and resistant (K1) strains of *Plasmodium falciparum* were determined as well as their toxicity against human embryonic kidney cells (HEK 293), hepatocellular carcinoma cells (Hep G2), B-lymphocyte cells (Raji) and human fibroblast cells (BJ). The compounds were prepared by linking the artemisinin and triazine pharmacophores with an aminoethylether chain using conventional and microwave assisted syntheses, and their structures were confirmed by NMR and HRMS techniques. All compounds were active against both strains of *P. falciparum* and were found to be non-toxic against all mammalian cell lines. They were overall more potent than chloroquine, irrespective of the *P. falciparum* strain considered. Compound **15**, featuring aniline and morpholine substituents on the triazine ring, was not only meaningfully more potent than chloroquine but was also found to possess activity comparable to those of artesunate and artemether against both malaria strains.

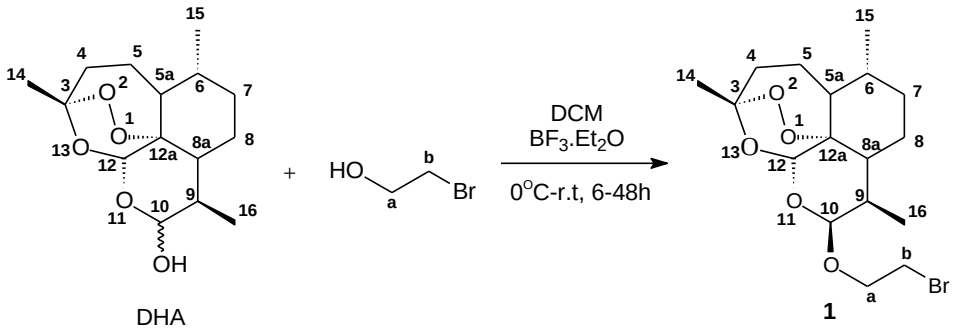
Keywords: Malaria, *Plasmodium falciparum*, dimers, analogs, microwave, artemisinin, triazine.

Despite decades of research aimed at eradicating malaria, the disease still threatens 6.6 billion people, with an approximate 655 000 people that die annually.[1] Past efforts to eliminate malaria was prevented by the parasite's notable ability to acquire resistance to the majority of drugs it is exposed to, rendering the use of chloroquine, sulfadoxine–pyrimethamine, mefloquine, amodiaquine, quinine and other drugs useless.[2] The artemisinin class of compounds showed great potential, but with unfortunate pharmacokinetic properties and resistance already being reported, the need for further research to obtain new antimalarial drugs is warranted.[3]

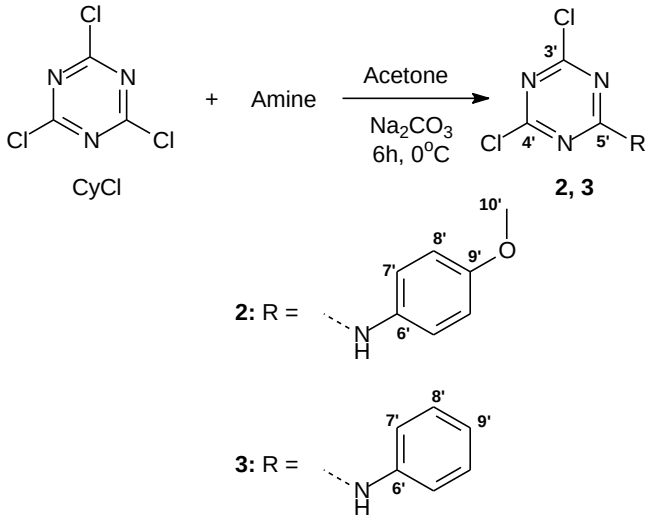
A proven method of overcoming resistance is the combination of two distinct pharmacophores into a single molecule. These hybrid molecules act as a "double edged sword" with a duel mechanism of action and often have the capability of withstanding resistance and to exhibit increased activity against a certain disease.[4-6] Another technique for avoiding resistance is by coupling the same pharmacophore to itself, thus forming a dimer, trimer, etc. It has been demonstrated that by forming a dimer, loss of activity due to resistance can be overcome and an increase in activity can be observed.[7-9]

Artemisinin dimers have been reported in the literature to have remarkable antimalarial and anticancer activity compared to artemisinin.[7,9-12] In addition to the reported increase in activity another advantage is the ability for a given drug to withstand resistance.[7,13]

In this article we report the synthesis and antimalarial activity of six dimeric artemisinin triazine hybrids consisting of two artemisinin pharmacophores attached *via* linkers to the triazine pharmacophore, as found in cycloguanil. We have previously reported the synthesis and antimalarial activity of seven monomeric artemisinin triazine hybrids.[14] The dimers were formed alongside the monomeric hybrids during the final step of the multi-step synthesis. Thus, the process ultimately delivered two product types, *viz.* the monomeric hybrids already reported[14], and the dimers in this article.

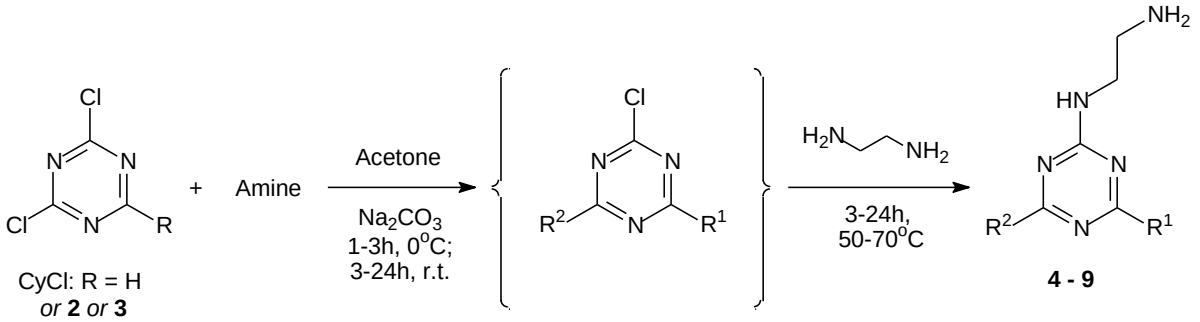


Scheme 1. Etheration of DHA yielding compound **1**.



Scheme 2. Monosubstitution of CyCl

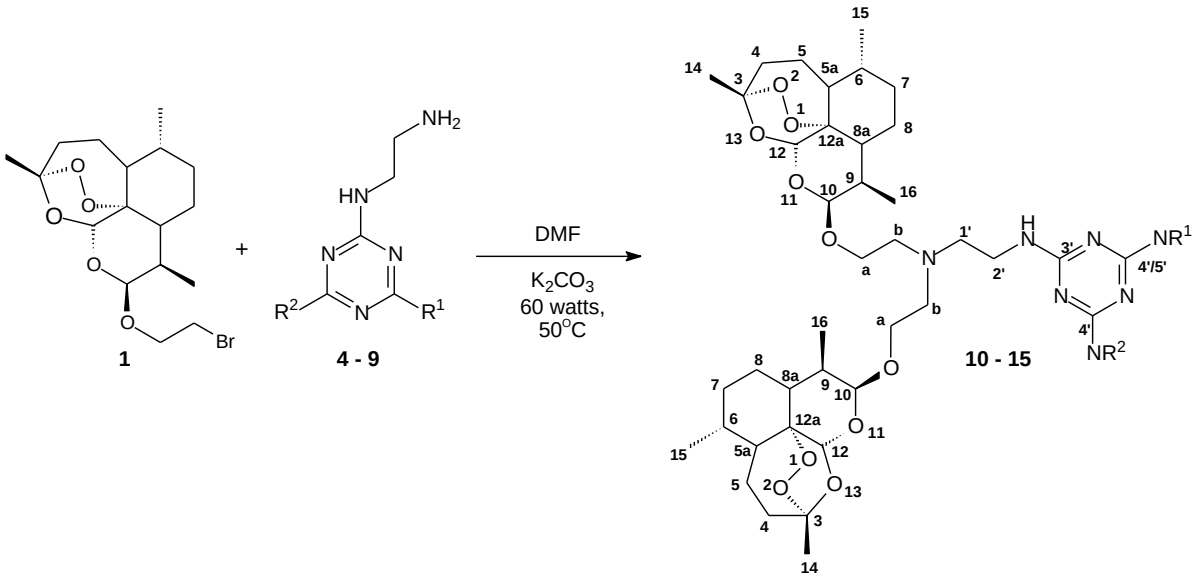
Dihydroartemisinin (DHA) was firstly converted to compound **1** by a reaction already reported (Scheme 1).[15] Monosubstituted cyanuric chloride (CyCl) compounds were then synthesised using the method described elsewhere yielding compounds **2** and **3** (Scheme 2).[14] Subsequently, compound **2**, **3** or unsubstituted CyCl was reacted with a given amine forming a disubstituted intermediate compound, followed by the *in situ* amination with ethylenediamine (EDA), which resulted in the formation of trisubstituted CyCl intermediates **4 – 9** (Scheme 3). The final compounds **10 – 15** were obtained by nucleophilic substitution reactions between **1** and the aforementioned primary amino-functionalised CyCl intermediates using microwave radiation (Scheme 4).

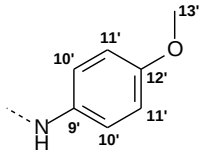
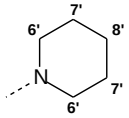
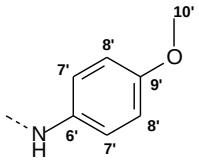
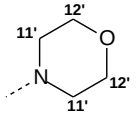
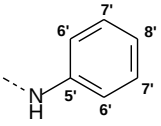
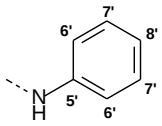


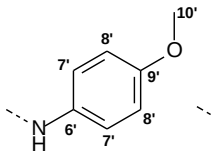
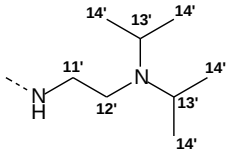
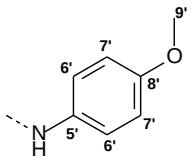
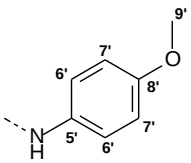
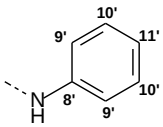
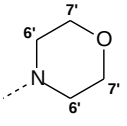
Scheme 3. Amination of CyCl, **2** and **3** (R^1 & R^2: **4 ≡ 10**; **5 ≡ 11**; **6 ≡ 12**; **7 ≡ 13**; **8 ≡ 14**; **9 ≡ 15**).

Compounds **1**, **10** - **15** were all in the 10β-isomer form as is confirmed by a coupling constant of J = 3.4 Hz between H-10 and H-9, and was previously determined by X-ray analysis.[16,17] Compounds **10** - **15** were isolated either as oils or solids, but were converted into oxalate salts

for stability and solubility reasons. Their structures were confirmed by both HRMS and NMR techniques. In compounds **4** – **9**, the secondary N-atom is rendered less nucleophilic due to resonance of the already disubstituted triazine ring, making it unavailable for substitution reactions.



Compound	R^1	R^2
10		
11		
12		

Compound	R^1	R^2
13		
14		
15		

Scheme 4. Synthesis of dimeric artemisinin-triazine hybrids **10** – **15**.

Subsequently, only the primary amine is involved in the substitution reactions leading to geminal dimers. Conversion of the secondary amine to a tertiary amine in the dimers, resulted in the ^{13}C NMR signals of both C-b and C-1' carbon atoms to shift from roughly δ 47 to 53 ppm, whilst the signal pertaining to C-2' stayed at δ 37 ppm. Similarly, the signals associated

with H-1' and H-b shifted from 3.10 to 3.50 whilst H-2' stayed roughly at the same position, indicating that the second coupling of compound **1** took place on the amine between C-b and C-1'.

The antimalarial activity and cytotoxicity of compounds **10** – **15** were tested using methods previously reported.[14] The EC_{50} values were determined against both the chloroquine sensitive (CQS) 3D7 and the chloroquine resistant (CQR) K1 strains of *Plasmodium falciparum*, whilst the cytotoxicity was determined against human embryonic kidney cells (HEK 293), hepatocellular carcinoma cells (Hep G2), B-lymphocyte cells (Raji) and human fibroblast cells (BJ).[14] Artesunate (AS), artemether (AM) and chloroquine (CQ) were used as reference drugs in the antimalarial assays whilst staurosporine was used in the cytotoxicity assays.[14] EC_{50} values 5 times higher or lower than that of the standard were considered as significantly different while those differing 10 times were considered as meaningfully different.

The *in vitro* antimalarial results showed that against the CQS strain only compound **10** were slightly less potent than CQ, with compounds **11** - **14** being more potent, and **15** being meaningfully more active. Compared to AS and AM, **15** was more potent than both these reference drugs. While all the other compounds were less potent than AS and AM, only compounds **10**, **13** and **14** showed a significant lower activity than AM versus the CQS strain. Against the CQR strain all compounds had meaningfully better activity than CQ. When comparing the synthesised compounds to AS and AM, compound **15** was slightly more potent than both. Whilst **11** – **14** were less potent than AS and AM, only compound **10** was significantly less active than AM.

Compound **15**, with an aniline and a morpholine moiety as substituents on the triazine ring, proved to be the compound with the highest activity against the 3D7 strain. Compound **11**, with a *p*-anisidine and a morpholine moiety on the triazine ring, is the synthesised compound with the second highest activity. Compounds **11** and **15** are analogous to one another, differing only on the *para* position of the aniline ring with a methoxy group. Although **11** is the second most active compound, **15** is five times more active. The methoxy group on the *para* position of the aniline ring thus led to a five-fold decrease in activity. Bigucci and co-workers found that the hydrogen-bonding properties, molecular size and shape, polarity, flexibility and the charge/ionization of a compound as a whole affect the absorption of the molecule. The observed decrease in activity might thus be as a result of the increased polarity resulting from the addition of the oxygen atom, decreasing the amount of compound capable of crossing the biological membrane and reaching its site of action.[18]

An interesting observation was that **11** and **15** were the only synthesised compounds that had calculated log P values below 5. Compounds **10**, **12**, **13** and **14** had calculated log P values of 5.8, 6.9, 7.0 and 6.8 respectively,[19] all with EC_{50} values higher than that of **11** and **15**.

Table 1. *In vitro* antimalarial activity of compounds **10 – 15** 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.

			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**
10	5.8	6.9 6.4	14.4	11.6	0.8	>27.1	>27.1	>27.1	>27.1	>1.9	>1.9	>1.9	>1.9
11	4.3	6.9 6.2	5.1	3.7	0.7	>28.5	>28.5	>28.5	>28.5	>5.6	>5.6	>5.6	>5.6
12	6.9	6.9 4.9	7.0	6.7	1.0	>21.0	>21.0	9.2	8.0	>3.0	>3.0	1.3	1.1
13	7.0	6.9 5.0	11.9	9.9	0.8	9.6	5.1	>26.2	>26.2	0.8	0.4	>2.2	>2.2
14	6.8	6.9 5.2	10.1	8.6	0.9	>27.6	>27.6	>27.6	>27.6	>2.7	>2.7	>2.7	>2.7
15	4.3	6.9 5.9	0.9	1.2	1.3	>27.7	>27.7	>27.7	>27.7	>30.8	>30.8	>30.8	>30.8
AS	3.0	4.3	3.2	2.8	0.9	>8.1	>8.1	>26.0	>26.0	>2.5	>2.5	>8.1	>8.1
AM	3.0	NA	1.8	2.3	1.3	>14.5	>14.5	>26.0	>26.0	>8.0	>8.0	>14.4	>14.4
CQ†	4.7	7.5 10.4	13.2	1670.9	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[19]; [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

When looking specifically at compounds **10** and **11**, this homologous pair differs from one another only in the oxygen atom in the *para* position of the cyclic substituent on the triazine moiety, causing a 3-fold difference in activity. The pK_a values of the compounds within this set are roughly the same, but the calculated log P value of **10** is higher than that of **11** (5.8 *vs.* 4.3). Thus, the compound with the oxygen atom has a lower EC_{50} and log P value. As noted in our previous article,[14] we believe that a log P value above 5, which is the preferred upper limit according to Lipinki's rule of five, leads to a decrease in activity.[20]

When comparing the activity of AS, AM and compounds **10** – **15** in relation to the CQS versus the CQR strain, a conservation of activity was observed.

Compounds **10**, **11**, **12** and **13** all had corresponding monomer compounds that have been reported in our previous article.[14] Against both the CQS and CQR strains, each dimer showed a slightly higher activity than their monomer counterpart[14], except compound **13**. The increase in activity can be explained by the fact that more artemisinin reaches the site of action.

The selectivity index (SI) showed that all of the synthesised compounds had a high degree of selectivity towards the parasitic cells, and was also found to be non toxic to the mammalian cells (Table 1).

In this study a series of six dimeric artemisinin triazine hybrids were successfully synthesised by linking the two pharmacophores with an aminoethylether chain. Against the CQS strain only compound **15** was meaningfully more active than chloroquine. Compared to AS and AM, all compounds proved to exhibit comparable potency except **10**, **13** and **14** that were significantly less potent than AM. Against the CQR strain, all compounds were significantly more potent than chloroquine. In comparison to AS and AM, all compounds had similar potency, expect **10** that were significantly less active than AM. Comparing compound **11** to **15**, it was found that a methoxy group on the aniline ring led to a fivefold decrease in activity possibly as a result of an increase in polarity. Compounds **10**, **11**, **12** and **13** all had corresponding monomer equivalents.[14] In each instance the dimer had a slightly higher activity except compound **13** that was less active than its matching monomer. None of the dimers showed mentionable toxicity against HEK 293, BJ, Raji or Hep G2 cells.

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22. *General procedure for the synthesis of 2-bromo-(10β-dihydroartemisinoxy) ethane, **1*** DHA derivative **1** has been synthesised and characterised before (Scheme 1).[15,21]
23. *General procedure for the monosubstitution of cyanuric chloride, **2, 3*** The synthesis of compounds **2** and **3** has been described elsewhere (Scheme 2).[14]
24. *Primary amino-functionalized triazines, **4** – **9*** To a solution of either CyCl or monosubstituted CyCl (**2**, **3**) dissolved in acetone at 0°C was added Na_2CO_3 and then the pertinent amine divided into four portions (Scheme 3). The reaction mixture was stir for 1 - 3 hours at 0°C, then for 3 - 24 hours at room temperature after which the reaction was stopped by removing the solvent *in vacuo*. The resulting deposit was dissolved in either EtOAc or dichloromethane (DCM) and washed with water, then purified by column chromatography

eluting with various ratios of MeOH, EtOAc, DCM, petroleum ether (PE) and NH_4OH. The resulting product was then used without further purification. Secondly, the above mentioned intermediate was dissolved in ethylenediamine (EDA) and heated with continuous stirring at 50 - 70°C for 3 - 24 hours. After completion, the reaction was quenched with ice cold water and extracted with EtOAc (3 x 100 ml) then purified by column chromatography eluting with various ratios of MeOH, DCM, and NH_4OH. The synthesis of compounds **4** – **7** have been described elsewhere.[14] *2-N-(2-aminoethyl)-4-N,6-N-bis(4-methoxyphenyl)-1,3,5-triazine-2,4,6-triamine,* ***8*** A solution of CyCl (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) was allowed to stir for 2 hours in an ice bath, then 5 hours at room temperature, followed by extraction with EtOAc. Purification with silica gel column chromatography eluting with EtOAc:PE (1:4) afforded 4.2 g of the intermediate as a brown powder. The intermediate (11.8 mmol, 4.2 g) with EDA (373.5 mmol, 25 ml, 32 eq.) at 60°C was allowed to stir for 24 hours. Extraction with EtOAc then purification with silica gel column chromatography eluting with MeOH:DCM:NH_4OH (1: 4: 0.2) afforded 3.2 g (71%) of **8** as a white powder. m.p. 150.1°C; $C_{19}H_{23}N_7O_2$. ^{1}H NMR (600 MHz, DMSO) 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, $CDCl_3$) 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'). COSY & HSQC (See annexure C). HRMS *m/z* $[M+H]^+$: 382.1959 (Calcd. for $C_{19}H_{23}N_7O_2$: 382.1923). *2-N-(2-aminoethyl)-6-(morpholin-4-yl)-4-N-phenyl-1,3,5-triazine-2,4-diamine,* ***9*** A solution of **3** (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) was allowed to stir for 1 hour in an ice bath, then 3 hours at room temperature, followed by extraction with EtOAc. Purification with silica gel column chromatography eluting with EtOAc:PE (1:4) afforded 2.2 g of the intermediate as a white powder. The intermediate (6.9 mmol, 2 g) with EDA (373.5 mmol, 25 ml, 54.1 eq.) at 60°C was allowed to stir for 5 hours. Extraction with EtOAc and purification with silica gel column chromatography eluting with MeOH:DCM:NH_4OH (1:9:0.5) afforded 1.8 g (85%) of **9** as a white powder. m.p. 95.6°C; $C_{15}H_{21}N_7O$. ^{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'). COSY & HSQC (See annexure C). HRMS *m/z* $[M+H]^+$: 316.1863 (Calcd. for $C_{15}H_{21}N_7O$: 316.1818).

25. *General procedure for the reaction between DHA-ethyl bromide* (**1**) *and the trisubstituted triazines,* ***10*** *–* ***15.*** The DHA derivative (**1**), triazine intermediates (**4** - **9**) and K_2CO_3 were dissolved in DMF. The reaction mixture was heated in a microwave reactor in bursts of 60 W and 50°C for 4 minutes at a time, cooling the reaction mixture to 0°C in between bursts. This was repeated until the reaction was complete (Scheme 4). Removal of the solvent *in vacuo*, dissolving 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. This reaction yielded both monomeric and dimeric compounds. *2-N-{2-[bis(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}-4-N-(4-methoxyphenyl)-6-(piperidin-1-yl)-1,3,5-triazine-2,4-diamine,* ***10*** The reaction between **1** (5.8 mmol, 2.28 g) and **4** (5.8 mmol, 2 g, 1 eq.) with K_2CO_3 (11.6 mmol, 1.6 g, 2 eq.) in DMF (50 ml) followed by purification with silica gel column chromatography eluting with MeOH:EtOAc:NH_4OH (1:29:0.5) yielded both the monomers and dimers, with the latter having a higher Rf value. Further purification of the dimer with silica gel column chromatography eluting with EtOAc:DCM:NH_4OH (1:1:0.5) and conversion of the resulting oil into the salt afforded 0.3 g (5 %) of **10**

as a white powder, m.p. 139.4°C; $C_{51}H_{77}N_7O_{11}$. ^{1}H NMR (600 MHz, $CDCl_3$) δ 7.45 (t, *J* = 18.7 Hz, 2H, H-10'), 6.79 (t, *J* = 22.0 Hz, 2H, H-11'), 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-6', H-13', 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-7', H-8'), 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 (Oxalic acid), 161.00 (C-5'), 156.51 (C-3'), 155.63 (C-4'), 153.37 (C-12'), 129.65 (C-9'), 122.98 (C-10'), 113.78 (C-11'), 104.33 (C-3), 102.17 (C-10), 87.89 (C-12), 80.69 (C-12a), 62.71 (C-a), 55.26 (C-13') 53.01 (C-1', C-b), 52.37 (C-5a), 45.55 (C-6'), 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-7'), 24.38 (C-5, C-8), 23.91 (C-8'), 20.29 (C-15), 12.77 (C-16). COSY & HSQC (See annexure C). HRMS *m/z* $[M+H]^+$: 964.5755 (Calcd. for $C_{51}H_{77}N_7O_{11}$: 964.5691). *2-N-{2-[bis(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-10yl]oxy} ethyl) amino]ethyl} 4-N(4-methoxyphenyl)-6 (morpholin-4-yl)-1, 3, 5-triazine-2, 4-diamine,* ***11*** The reaction between **1** (2.9 mmol, 1.14 g) and **5** (2.9 mmol, 1 g, 1 eq.) with K_2CO_3 (5.8 mmol, 0.8 g, 2 eq.) in DMF (50 ml) followed by purification with silica gel column chromatography eluting with MeOH:EtOAc:PE:NH_4OH (1:10:4:0.5) yielded both the mono- and dimers, with the latter having a higher Rf value. Further purification of the dimer with silica gel column chromatography eluting with EtOAc:PE:NH_4OH (39:1:0.5) and conversion of the resulting oil into the salt afforded 0.1 g (4 %) of **11** as a white powder, m.p. 145.3°C; $C_{50}H_{75}N_7O_{12}$. ^{1}H 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 (m, 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 (Oxalic acid), 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). COSY & HSQC (See annexure C). HRMS *m/z* $[M+H]^+$: 966.5553 (Calcd. for $C_{50}H_{75}N_7O_{12}$: 966.5483). *2-N-{2-[bis(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}-4-N,6-N-diphenyl-1,3,5-triazine-2,4,6-triamine,* ***12*** The reaction between **1** (2.3 mmol, 0.9 g) and **6** (2.3 mmol, 0.724 g, 1 eq.) with K_2CO_3 (4.6 mmol, 0.63 g, 2 eq.) in DMF (25 ml) followed by purification with silica gel column chromatography eluting with MeOH:EtOAc:NH_4OH (1:29:0.5) yielded both the mono- and dimers, with the latter having a higher Rf value. Further purification of the dimer with silica gel column chromatography eluting with EtOAc:DCM (1:1) and conversion of the resulting oil into the salt afforded 0.1 g (2.5 %) of **12** as an off-white powder, m.p. 109.3°C; $C_{51}H_{71}N_7O_{10}$. ^{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 (t, *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-8 β), 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). COSY & HSQC (See annexure C). HRMS *m/z* $[M+H]^+$: 942.5322 (Calcd. for $C_{51}H_{71}N_7O_{10}$: 942.5262). *2-N-*

{2-[bis(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}-4-N-{2-[bis(propan-2-yl)amino]ethyl}-6-N-(4-methoxyphenyl)-1,3,5-triazine-2,4,6-triamin, ***13*** The reaction between **1** (5.0 mmol, 1.94 g) and **7** (5.0 mmol, 2.0 g, 1 eq.) with K_2CO_3 (9.94 mmol, 1.37 g, 2 eq.) in DMF (25 ml) followed by successive purification firstly with silica gel column chromatography eluting with MeOH:DCM:NH_4OH (1:9:0.5) and then with MeOH:EtOAc:NH_4OH(1:14:0.5) yielded both the mono- and dimers, with the latter having a higher Rf value. Further purification of the dimer product with silica gel column chromatography eluting with MeOH:EtOAc:NH_4OH (1:49:0.5) and conversion of the resulting oil into the salt afforded 0.4 g (7 %) of **13** as a white powder, m.p. 141.9°C; $C_{54}H_{86}N_8O_{11}$. ^{1}H NMR (600 MHz, DMSO) 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) 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). COSY & HSQC (See annexure C). HRMS *m/z* $[M+H]^+$: 1023.6457 (Calcd. for $C_{54}H_{86}N_8O_{11}$: 1023.6426). *2-N-{2-[bis(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}-4-N,6-N-bis(4-methoxyphenyl)-1,3,5-triazine-2,4,6-triamine,* ***14*** The reaction between **1** (2.62 mmol, 1.02 g) and **8** (2.62 mmol, 1.0 g, 1 eq.) with K_2CO_3 (5.24 mmol, 0.724 g, 2 eq.) in DMF (25 ml) followed by successive purification firstly with silica gel column chromatography eluting with EtOAc and secondly with MeOH:DCM (1:14) and conversion of the resulting oil into the salt afforded 0.1 g (3 %) of **14** as a white powder, m.p. 147.4°C; $C_{53}H_{75}N_7O_{12}$. ^{1}H NMR (600 MHz, DMSO) δ 7.62 (s, 4H, H-6'), 6.83 (s, 5H, H-7'), 5.34 (s, 2H, H-12), 4.70 (s, 4H, H-10), 3.88 (d, *J* = 51.9 Hz, 3H, H-aα), 3.79 – 3.40 (m, 11H, H-H-aβ, H-9', H-2'), 3.16 (d, *J* = 55.0 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) δ 165.59 (C-3'), 163.77 (C-4'), 162.69 (Oxalic acid), 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). COSY & HSQC (See annexure C). HRMS *m/z* $[M+H]^+$: 1002.5512 (Calcd. for $C_{53}H_{75}N_7O_{12}$: 1002.5484). *2-N-{2-[bis(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}-6-(morpholin-4-yl)-4-N-phenyl-1,3,5-triazine-2,4-diamine,* ***15*** The reaction between **1** (3.17 mmol, 1.2 g)and **9** (3.17 mmol, 1.0 g, 1 eq.) with K_2CO_3 (6.34 mmol, 0.876 g, 2 eq.) in DMF (40 ml) followed by successive purification firstly with silica gel column chromatography eluting with MeOH:EtOAc:NH_4 (1:14:0.5) and secondly with EtOAc:PE:NH_4OH (39:1:0.5) and conversion of the resulting oil into the salt afforded 0.3 g (11 %) of **15** as a white powder, m.p. 143.7°C; $C_{49}H_{73}N_7O_{11}$. ^{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 (ddd, *J* = 24.4, 20.7, 17.9 Hz, 6H, H-1', H-b), 2.73 – 2.54 (m, 2H, H-9), 2.32 (td, *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 (ddd, *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 (Oxalic acid), 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). COSY & HSQC (See annexure C). HRMS *m/z* $[M+H]^+$: 936.5439 (Calcd. for $C_{49}H_{73}N_7O_{11}$: 936.5378).