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Synthesis and Antiplasmodial Activity of EG-Artemisinin Ethers and Artemisinin–Quinoline Hybrids

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ABSTRACT

The aim of this study was to synthesize a series of ethylene glycol (EG) ethers and quinoline hybrids of the antimalarial drug artemisinin and to evaluate their antimalarial activity *in vitro* against *Plasmodium falciparum* strains. The ethers were synthesized in a one-step process by coupling ethylene glycol (EG) moieties of various chain lengths to carbon 10 of dihydroartemisinin, while the artemisinin-quinoline hybrids were obtained by condensation of dihydroartemisinin with different amine-functionalized quinoline moieties. For solubility reasons, part of the hybrids were converted to oxalate salts upon reaction of the free bases with oxalic acid. All the synthesized compounds were tested against chloroquine (CQ) susceptible (CQS) D10 and chloroquine resistant (CQR) Dd2 *Plasmodium falciparum* strains. The IC_{50} values revealed that all the ethers were active against both strains but less potent than artemether irrespective of the strain. However, they were more active than CQ against the resistant strain. Ether 8 featuring three EO units was the most active of all ethers. It showed activity similar to that of CQ against D10 and much more potency than CQ against Dd2 strain (IC_{50}, 0.023 *vs.* 0.473 nM). The hybrids and their salts were also all active against both strains. Hybrid 19 which possessed an isopropyl linker and its oxalate salt 19a were the most active against the Dd2 strain. They were more potent than CQ (IC_{50}, 0.009 and 0.011 *vs.* 0.255 nM, respectively).

KEYWORDS

Artemisinin (ART), dihydroartemisinin (DHA), artemether (ARM), chloroquine (CQ), malaria, *Plasmodium falciparum*, ethylene glycol) (EG), ethylene oxide (EO), hybrid.

1. Introduction

Malaria remains a major cause of morbidity and death in tropical countries all over the world and a substantial number of people are exposed to the risk of contracting this deadly disease each year. With approximately 243 million cases and 863 000 attributed deaths reported globally in 2009, malaria is one of the most severe infectious diseases, primarily affecting the world's most disadvantaged populations[1].

Malaria chemotherapy based on standard drugs such as quinine especially is becoming more and more difficult because the malaria parasites, *Plasmodium falciparum*, causing the disease have developed widespread resistance against them. This warrants the search for a new classes of natural and synthetic antimalarial drugs.

Artemisinin and its derivatives (Fig. 1) showed a rapid onset of action, low toxicity and high antimalarial activity against both drug-resistant and drug-sensitive malaria, in early clinical studies[2]. The practical values of these antimalarial agents, nevertheless, are impaired by their poor solubility in oil and water, poor oral bioavailability, high rate of parasite recrudescence after treatment[3] and short plasma half-life[4].

In order to overcome these pharmacokinetic deficiencies, a program aiming at modifying the chemical structure of artemisinin was launched in 1976, which resulted in a number of new analogues with improved efficacy and increased solubility: oil-soluble artemether and arteether 5 and water-soluble sodium artesunate[5]. Artemether is the methyl ether derivative of artemisinin and arteether the ethyl ether derivative; these compounds are lipophilic and more potent than artemisinin, but still have a short plasma half-life. Artemisinin, dihydroartemisinin **1**, artemether and arteether are all poorly water-soluble compounds, which results in slower and incomplete absorption of these drugs into the systemic circulation; sodium artesunate is much more hydrophilic which leads to better absorption[6]. However, the usefulness of sodium artesunate in the treatment of cerebral malaria and multidrug-resistant *P. falciparum* is offset by problems associated with its instability in aqueous medium[7], the high rate of recrudescence and the drug's extremely short plasma half-life[8].

The field for discovering new antimalarial compounds is still widely open. Gamo and coworkers[9] screened nearly 2 million compounds from GlaxoSmithKline's chemical library for activity against *P. falciparum* and found 13 533 that inhibit parasite growth by at least 80 % at 2 nM concentration. More than 8000 also showed potent activity against the multidrug resistant strain Dd2. They are of the opinion that these thousands of chemicals could be starting points for antimalarial lead identification.

Pegylation, generally described as the molecular attachment of poly(ethylene glycol)s (PEG) with different molecular weights to active drug molecules, is one of the most promising and extensively studied strategies with the goal of improving the pharmacokinetic behaviour of therapeutic drugs[10]. The main pharmacokinetic outcomes of pegylation are summarized as changes occurring in overall circulation life-span, tissue distribution pattern, and elimination pathway of the parent drug[11]. The attachment of a PEG moiety to drug molecules increases the

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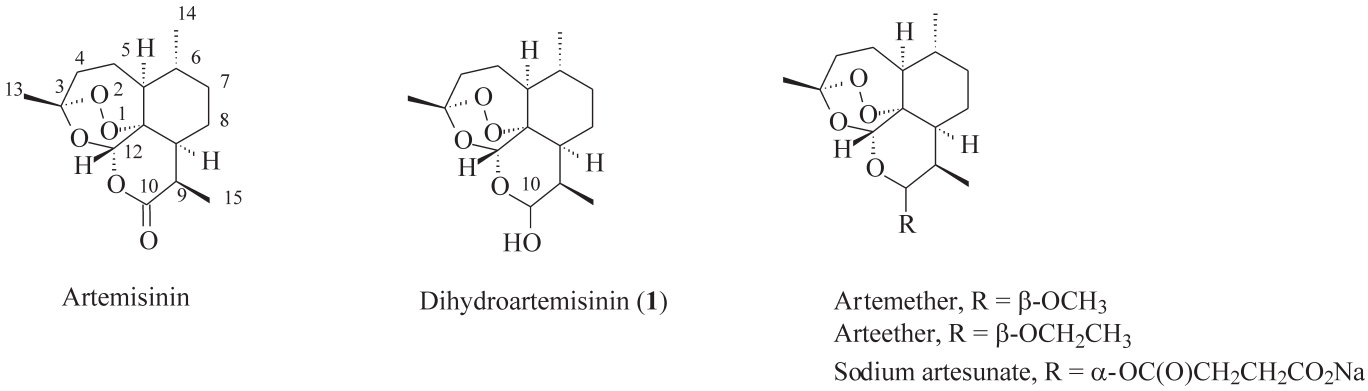


Figure 1 Artemisinin and its derivatives.

overall size of the parent drug and the circulation half-life of PEGs increase with the increase in molecular weight[12]. Pegylated drugs are also more stable over a range of pH and temperature changes[13] compared with their unpegylated counterparts. Consequently, pegylation confers on drugs a number of properties that are likely to result in a number of clinical benefits, including sustained blood levels that enhance effectiveness, fewer adverse reactions, longer shelf-life and improved patient convenience[14].

In search for stable, more water-soluble, highly potent, long acting artemisinin derivatives, we modified the artemisinin molecule by introducing EG moieties at its C10-position through ether linkage. Furthermore, an emerging strategy within medicinal chemistry and drug discovery is the chemical combination of two distinct pharmacophores into a single molecule, the so-called hybrid. The hybrid molecule offers a simpler and more effective way of delivering two drugs, especially when differences like elimination times occur. A careful choice of the linker can allow the intact hybrid to dissociate into its individual components or to remain fixed when metabolically resistant linker units are chosen. The first case is particularly advantageous if the individual components have different sites of action within the cell. In the second case, especially with respect to overcoming resistance, it is desirable for the hybrid to resist metabolic cleavage[15].

The success of quinoline-based antimalarials were based on their excellent clinical efficacy, limited host toxicity and further so on. However, their use has been seriously eroded in recent years, mainly as a result of the development and spread of parasite resistance[16]. Despite this, identification of hybrid 4-aminoquinolines having multiple targets is a hope of generating effective antimalarial chemotherapy[17].

Building on this information, we synthesized artemisinin-quinoline hybrids and evaluated their antiplasmodial activity in similar conditions as for the EG ethers of artemisinin.

We herein report the synthesis and *in vitro* activity of both artemisinin derivative-types.

2. Experimental Procedures

2.1. Instrumentation

Thin-layer chromatography (TLC) was performed using silica gel plates ($60F_{254}$ Merck). Preparative flash column chromatography was carried out on silica gel (230–240 mesh, G60 Merck) and silica gel 60 (70–230 mesh ASTM, Fluka).

The ^{1}H and ^{13}C NMR spectra were recorded on a Bruker Avance III 600 spectrometer in deuterated chloroform ($CDCl_3$) using tetramethylsilane (TMS) as internal standard. The splitting pattern abbreviations are as follows: s (singlet), d (doublet), dd (doublet of doublets), δ (doublet of doublet of doublets), dt (doublet of triplets), t (triplet), td (triplet of doublets), tt (triplet of triplet) and m (multiplet).

The low-resolution fast atom bombardment (FAB) mass spectra (MS) were recorded for the artemisinin–EG ethers on a VG70SE mass spectrometer purchased from Thermo Fisher Scientific Inc. (Waltam, MA, USA), and using a xenon atom beam at 8 kV and a 3-nba matrix in all cases. Positive ions (MH+) and (MNa+) were recorded.

The artemisinin-quinoline hybrids were run on the Thermo Finnigan (California, US) LXQ linear ion trap using APCI as ionization mode in positive mode with Vaporizer temp of 340 °C, capillary temperature of 180 °C and discharge Current at 10 uA.

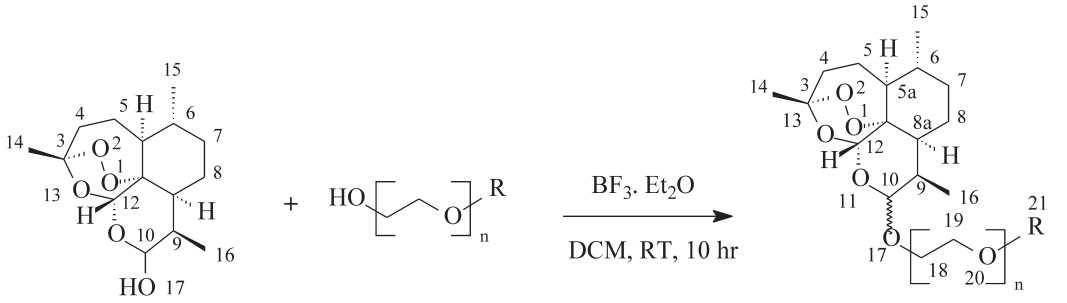
2.2. Synthetic Procedures

2.2.1. *Synthesis of EG Ethers of Artemisinin* **3–8**

The synthesis of EG ethers of artemisinin (Scheme 1) was achieved by using with slight modifications the general method reported by Li *et al.*[18], and described as follows: to a solution of DHA (1) (2.0 g, 7 mmol) and methoxy(ethylene glycol) (MEG) or ethoxy(ethylene glycol) (EEG) (14 mmol, 2.0 eq. relative to DHA) dissolved in 50 mL of anhydrous dichloromethane (DCM, CH_2Cl_2), was added boron trifluoride diethyl etherate (BF_3 Et_2O) (1.0 mL) portion wise at 0 °C. The mixture was stirred at 0 °C for 0.5 h, then at room temperature (RT) for 10 h. The progress of the reaction was monitored by thin layer chromatography (TLC). After completion, the reaction mixture was washed successively with a saturated $NaHCO_3$ solution, water and brine. The organic layer was dried over $MgSO_4$ and evaporated to dryness under reduced pressure. The resultant oil was purified by flash chromatography eluting with DCM:EtOAc (ratios as described below) as mobile phase. All the synthesized compounds were oils, and failed to crystallize. ^{1}H and ^{13}C NMR chemical shifts as well as FAB-MS data of compounds **3–8** are reported.

10β-(2-Methoxyethoxy)-dihydroartemisinin **3** *(R = CH_3, n = 1)*

Ether **3** as obtained as clear oil in 38 % yield after purification by flash silica gel column chromatography eluting with DCM:EtOAc (20:1). δ_H (600 MHz, $CDCl_3$): 5.41 (1H, s, H-12), 4.80 (1H, d, *J* 4.8 Hz, H-10), 3.95–3.87 (1H, m, H-18a), 3.58–3.46 (3H, m, H-18b and H-19), 3.37–3.31 (3H, m, OCH_3, H-21), 2.64–2.54 (1H, m, H-8a), 2.34 (1H, m, H-9), 2.00 (1H, m, H-5a), 1.88–1.57 (5H, m, H-5, -6 and H-8), 1.51–1.38 (4H, m, H-4 and H-7), 1.35–1.15 (3H, m, H-14) and ppm 0.95–0.82 (6H, m, H-15 and H-16); δ_C (600 MHz, $CDCl_3$): 103.04 (C-10), 102.89 (C-3), 88.98 (C-12), 81.60 (C-12a), 71.64 (C-19), 67.53 (C-18), 58.94 (C-21), 51.83 (C-5a), 46.52 (C-8a), 39.45 (C-9), 37.16 (C-4), 36.59 (C-7), 34.47 (C-6), 31.48 (C-14), 25.99 (C-5), 24.64 (C-8), 20.11 (C-15) and ppm 19.46 (C-16);



Dihydroartemisinin **1** EG **2** Artemisinin - EG ethers **3** - **8**

Compound	R	n
3	CH_3	1
4	CH_3	2
5a	CH_3	3
5b	CH_3	3
6a	CH_2CH_3	1
6b	CH_2CH_3	1
7	CH_2CH_3	2
8	CH_2CH_3	3

Scheme 1
Synthesis of artemisinin-EG ethers.

$C_{18}H_{30}O_6$ (M_w 342.43, calc.); FAB *m/z*: 343.3 (MH+, 8 %), 365.4 (MNa+, 4 %).

10β-[2-(2-Methoxyethoxy) ethoxy]-dihydroartemisinin **4** *(R = CH_3, n = 2)*

Derivative **4** was purified by flash silica column chromatography eluting with DCM:EtOAc (15:1) to produce light yellow oil: 1.28 g (47 %) yield. δ_H (600 MHz, $CDCl_3$): 5.41 (1H, s, H-12), 4.80 (1H, d, *J* 2.4 Hz, H-10), 3.95–3.87 (1H, m, H-18a), 3.58–3.46 (7H, m, H-18b, -19, -21 and H-22), 3.37–3.31 (3H, s, OCH_3, H-24), 2.64–2.54 (1H, m, H-8a), 2.34 (1H, dd, *J* 2.4 and14.2 Hz, H-9), 2.00 (1H, d, *J* 14.4 Hz, H-5a), 1.88–1.57 (5H, m, H-5, -6 and H -8), 1.51–1.38 (4H, m, H-4 and H-7), 1.35–1.15 (3H, m, H-14) and ppm 0.95–0.82 (6H, m, H-15 and H-16); δ_C (600 MHz, $CDCl_3$): 104.10 (C-10), 101.99 (C-3), 87.91 (C-12), 81.13 (C-12a), 71.95 (C-22), 70.52 (C-19), 70.40 (C-21), 67.39 (C-18), 59.03 (C-24), 52.60 (C-5a), 44.41 (C-8a), 37.36 (C-9), 36.40 (C-4), 34.60 (C-7), 30.82 (C-6), 26.16 (C-14), 24.66 (C-5), 24.48 (C-8), 20.25 (C-15) and ppm 12.87 (C-16); $C_{20}H_{34}O_7$ (M_w 386.48, calc.); FAB *m/z*: 387.3 (MH+, 12 %), 409.3 (MNa+, 11 %).

10β-{2-[2-(2-Methoxyethoxy)ethoxy]ethoxy}-dihydroartemisinin **5a** *(R = CH_3, n = 3)*

Purification by silica gel flash chromatography eluting with DCM:EtOAc (10:1) afforded Ether **5a** as yellowish oil in 48 % yield. δ_H (600 MHz, $CDCl_3$): 5.36 (1H, s, H-12), 4.76 (1H, d, *J* 4.3 Hz, H-10), 3.91–3.82 (1H, m, H-18a), 3.62–3.48 (11H, m, H-18b, -19, -21, -22, -24 and H-25), 3.31 (3H, s, H-27), 2.64–2.55 (1H, m, H-8a), 2.29 (1H, dd, *J* 4.3 and 14.1 Hz, H-9), 1.96 (1H, d, *J* 14.4 Hz, H-5a), 1.83–1.65 (4H, m, H-6 and H-8), 1.55 (2H, d, *J* 12.9, H-5), 1.43–1.34 (4H, m, H-4 and H-7), 1.29–1.11 (3H, m, H-14) and ppm 0.86 (6H, dd, *J* 6.8 and 23.3 Hz, H-15 and H-16); δ_c (600 MHz, $CDCl_3$): 103.96 (C-10), 102.05 (C-3), 87.90 (C-12), 81.12 (C-12a), 71.92 (C-25), 70.68 (C-19), 70.54 (C-21), 70.50 (C-22), 70.25 (C-24), 67.39 (C-18), 58.94 (C-27), 52.60 (C-5a), 44.41 (C-8a), 37.32 (C-9), 36.40 (C-4), 34.61 (C-7), 30.84 (C-6), 26.16 (C-14), 24.66 (C-5), 24.47 (C-8), 20.41 (C-15) and ppm 12.87 (C-16); $C_{22}H_{38}O_8$ (M_w 430.58, calc.); FAB *m/z*: 431.4 (MH+, 5 %), 453.4 (MNa+, 25 %).

10α-{2-[2-(2-Methoxyethoxy)ethoxy] ethoxy}-dihydroartemisinin **5b** *(R = CH_3, n = 3)*

Ether **5b** was obtained as yellow oil 12 % yield after purification on flash silica gel eluting with DCM:EtOAc (10:1). δ_H (600 MHz, $CDCl_3$): 5.26 (1H, s, H-12), 4.44 (1H, d, *J* 9.2 Hz, H-10), 3.97 (1H, t, *J* 8.7 Hz, H-18a), 3.59 (9H, dd, *J* 6.2 and 16.2 Hz, H-18b, -19, -21, -22 and H-24), 3.49–3.46 (m, 2H, H-25), 3.31 (3H, s, H-27), 2.32 (1H, δ, *J* 5.0, 13.5 and 16.7 Hz, H-8a), 1.95 (1H, d, *J* 9.2 Hz, H-9), 1.80 (1H, d, *J* 13.6 Hz, H-5a), 1.68–1.61 (4H, m, H-6 and H-8), 1.61 (1H, d, *J* 13.1 Hz, H-5), 1.50–1.35 (4H, m, H-4 and H-7), 1.27–1.17 (3H, m, H-14) and ppm 0.95–0.81 (6H, m, H-15 and H-16); δ_C (600 MHz, $CDCl_3$): 104.22 (C-10), 100.31 (C-3), 91.10 (C-12), 80.40 (C-12a), 71.90 (C-25), 70.86 (C-19), 70.71 (C-21), 70.57 (C-22), 70.42 (C-24), 68.13 (C-18), 58.93 (C-27), 51.53 (C-5a), 45.34 (C-8a), 37.32 (C-9), 36.28 (C-4), 34.28 (C-7), 32.49 (C-6), 26.01 (C-14), 24.67 (C-5), 22.21 (C-8), 20.25 (C-15) and ppm 12.55 (C-16); $C_{22}H_{38}O_8$ (M_w 430.58, calc.); FAB *m/z*: 431.1 (MH+, 5 %), 453.1 (MNa+, 11 %).

10β-(2-Ethoxyethoxy)-dihydroartemisinin **6a** *(R = CH_2-CH_3, n = 1)*

Ether **6a** was afforded in 35 % yield as clear oil after purified by flash silica gel column chromatography eluting with DCM:EtOAc (15:1). δ_H (600 MHz, $CDCl_3$): 5.41 (1H, s, H-12), 4.93 (1H, d, *J* 4.5 Hz, H-10), 3.95–3.90 (1H, m, OCH-CH_2-O, H-18a), 3.62–3.45 (5H, m, H-18b, -19 and H-21), 2.29–2.20 (1H, m, H-8a), 1.99 (1H, dd, *J* 4.5 and 42.6 Hz, H-9), 1.74–1.67 (1H, m, H-5a), 1.58–1.48 (3H, m, H-22), 1.39–1.33 (5H, m, H-5, -6 and H-8), 1.29–1.2 (4H, m, H-4 and H-7), 1.13–1.0 (3H, m, H-14) and ppm 0.95–0.89 (6H, m, H-15 and H-16); δ_C (600 MHz, $CDCl_3$):

102.95 (C-10),102.85 (C-3), 88.80 (C-12), 81.46 (C-12a), 69.45 (C-19), 67.51 (C-21), 66.36 (C-18), 51.80 (C-5a), 46.32 (C-8a), 39.45 (C-9), 37.15 (C-4), 36.39 (C-7), 34.45 (C-6), 31.38 (C-14), 25.82 (C-5), 24.58 (C-8), 19.97 (C-15), 19.40 (C-16) and ppm 15.08 (C-22); $C_{19}H_{32}O_6$ (M_w 356.45, calc.); FAB *m/z*: 357.4 (MH+, 10 %), 379.3 (MNa+, 7 %).

10α-(2-Ethoxyethoxy)-dihydroartemisinin **6b** *(R = CH_2-CH_3, n = 1)*

Ether **6b** was afforded in 17 % yield as clear oil after purified by flash silica gel column chromatography eluting with DCM:EtOAc (15:1). δ_H (600 MHz, $CDCl_3$): 5.27 (1H, s, H-12), 4.45 (1H, d, *J* 9.3 Hz, H-10), 3.97 (1H, dd, *J* 10.7, 4.3 Hz, H-18a), 3.60–3.46 (5H, m, H-18b, -19 and H-21), 2.55 (1H, d, *J* 3.3 Hz, H-8a), 2.33 (1H, δ, *J* 5.7, 9.3 and 28.3 Hz, H-9), 2.00–1.90 (1H, m, H-5a), 1.85–1.51 (3H, m, H-22), 1.51–1.32 (4H, m, H-4 and H-8), 1.32–1.07 (5H, m, H-5, -6 and H-7) and ppm 1.00–0.75 (9H, m, H-14, -15 and H-16); δ_C (600 MHz, $CDCl_3$): 104.23 (C-10), 100.35 (C-3), 91.16 (C-12), 80.40 (C-12a), 69.95 (C-19), 68.13 (C-21), 66.48 (C-18), 51.52 (C-5a), 45.34 (C-8a), 37.33 (C-9), 36.28 (C-4), 34.28 (C-7), 32.46 (C-6), 26.00 (C-14), 24.67 (C-5), 22.22 (C-8), 20.25 (C-15), 15.11 (C-16) and ppm 12.40 (C-22); $C_{19}H_{32}O_6$ (M_w 356.45, calc.); FAB *m/z*: 357.4 (MH+, 10 %), 379.3 (MNa+, 8 %).

10β-[2-(2-Ethoxyethoxy) ethoxy]-dihydroartemisinin **7**
(R = CH_2-CH_3, n = 2)

Ether **7** was obtained as light yellow oil 48 % yield after purification on flash silica gel eluting with DCM:EtOAc (10:1), $C_{21}H_{36}O_7$; δ_H (600 MHz, $CDCl_3$): 5.37 (1H, s, H-12), 4.76 (1H, d, *J* 4.6 Hz, H-10), 3.92–3.86 (1H, m, H-18a), 3.62–3.45 (9H, m, H-18b, -19, -21, -22 and H-24), 2.55 (1H, dd, *J* 8.6 and 19.4 Hz, H-8a), 1.96 (1H, d, *J* 14.5 Hz, H-9), 1.77 (1H, dd, *J* 11.6 and 28.0 Hz, H-5a), 1.70–1.54 (3H, m, H-25), 1.44–1.35 (4H, m, H-4 and H-8), 1.26 (1H, d, *J* 5.7 Hz, H-6), 1.16 (4H, dt, *J* 6.0 and 13.9 Hz, H-5 and H-7) and ppm 0.90–0.82 (9H, m, H-14, -15 and H-16); δ_C (600 MHz, $CDCl_3$): 103.90 (C-10), 101.99 (C-3), 87.91 (C-12), 81.14 (C-12a), 70.52 (C-19), 70.27 (C-22), 69.94 (C-21), 67.39 (C-24), 66.65 (C-18), 52.56 (C-5a), 44.57 (C-8a), 37.34 (C-9), 36.41 (C-4), 34.77 (C-7), 30.82 (C-6), 26.17 (C-14), 24.67 (C-5), 24.33 (C-8), 20.24 (C-15), 15.11 (C-16) and ppm 12.97 (C-25); $C_{19}H_{32}O_6$ (M_w 400.51, calc.); FAB *m/z*: 401.5 (MH+, 8 %), 426.4 (MNa+, 7 %).

10β-{2-[2-(2-ethoxyethoxy)ethoxy]ethoxy}-dihydroartemisinin **8**
(R = CH_2-CH_3, n = 3)

Ether **8** as obtained as clear oil in 51 % yield after purification by flash silica gel column chromatography eluting with DCM:EtOAc (5:1). δ_H (600 MHz, $CDCl_3$): 5.35 (1H, s, H-12), 4.75 (1H, d, *J* 4.4 Hz, H-10), 3.90–3.83 (1H, m, H-18a), 3.60–3.52 (13H, m, H-18b, -19, -21, -22, -24, -25 and H-27), 2.54 (1H, d, *J* 3.4 Hz, H-8a), 1.93(1H, dd, *J* 4.4 and 13.4 Hz, H-9), 1.73 (1H, δ, *J* 8.8, 22.1 and 41.2 Hz, H-5a), 1.54–1.48 (3H, m, H-28), 1.40–1.35 (4H, m, H-4 and H-8), 1.25 (1H, d, *J* 5.7 Hz, H-6), 1.2 -1.0 (4H, m, H-5 and H-7) and ppm 0.90–0.82 (9H, m, H-14, -15 and H-16); δ_C (600 MHz, $CDCl_3$): 104.00 (C-10), 102.06 (C-3), 87.81 (C-12), 81.09 (C-12a), 70.65 (C-19), 70.59 (C-25), 70.55 (C-24), 70. 47 (C-21), 69.78 (C-22), 67.37 (C-27), 66.67 (C-18), 52.62 (C-5a), 44.42 (C-8a), 37.38 (C-9), 36.38 (C-4), 34.66 (C-7), 30.88 (C-6), 26.13 (C-14), 24.64 (C-5), 24.30 (C-8), 20.33 (C-15), 15.15 (C-16) and ppm 12.94 (C-28); $C_{23}H_{40}O_8$ (M_w 444.56, calc.); FAB *m/z*: 467 (MNa+, 38 %).

2.2.2. *Synthesis of Artemisinin-Quinoline Hybrids* **16–21**

The target hybrids were obtained in a three-step process starting from commercially available dihydroartemisinin and 4,7-dichloroquine.

In the first step, amine-functionalized quinolines **10–15** were prepared by condensation of various diamines with 4,7-dichloroquinoline (Scheme 2) according to a method reported by N'Da *et al.*[19].

7-Chloro-4-(1,2-diaminoethyl) quinoline **10**

Off-white crystals; δ_H (600 MHz, CD_3OD): 8.35 (1H, d, *J* 5.6 Hz, H-2′), 8.10 (1H, d, *J* 9.0 Hz, H-5′), 7.76 (1H, d, *J* 2.1 Hz, H-8′), 7.39 (1H, dd, *J* 2.1 and 9.0 Hz, H-6′), 6.55 (1H, d, *J* 5.6 Hz, H-3′), 3.43 (2H, t, *J* 6.4 Hz, H-3′′) and ppm 2.97 (2H, t, *J* 6.4 Hz, H-4′′); δ_C (600 MHz, CD_3OD): 152.83 (C-2′), 149.68 (C-4′), 136.34 (C-7′), 127.62 (C-8′), 124.34 (C-5′), 118.82 (C-6′), 98.71 (C-3′), 46.37 (C-3′′) and ppm 40.88 (C-4′′).

7-Chloro-4-(1,3-diaminopropyl) quinoline **11**

Off-white crystals; δ_H (600 MHz, CD_3OD): 8.33 (1H, d, *J* 5.6 Hz, H-2′), 8.05 (1H, d, *J* 9.0 Hz, H-5′), 7.75 (1H, d, *J* 2.0 Hz, H-8′), 7.37 (1H, dd, *J* 2.1 and 9.0 Hz, H-6′), 6.50 (1H, d, *J* 5.6 Hz, H-3′), 3.40 (2H, t, *J* 7.0 Hz, H-3′′), 2.79 (2H, t, *J* 7.1 Hz, H-5′′) and ppm 1.89 (2H, m, H-4′′); δ_C (600 MHz, CD_3OD): 152.68 (C-2′), 149.67 (C-4′), 136.29 (C-7′), 127.61 (C-8′), 125.96 (C-5′), 124.28 (C-6′), 99.65 (C-3′), 41.73 (C-5′′), 40.28 (C-3′′) and ppm 32.03 (C-4′′).

7-Chloro-4-(1,4-diaminophenyl) quinoline **12**

Dark brown crystals; δ_H (600 MHz, CD_3OD): 8.26 (1H, d, *J* 5.6 Hz, H-2′), 8.23 (1H, d, *J* 9.0 Hz, H-5′), 7.80 (1H, d, *J* 2.1 Hz, H-8′), 7.43 (1H, dd, *J* 2.1 and 9.0 Hz, H-6′), 7.08 (2H, d, *J* 8.5 Hz, H-3′′), 6.81 (2H, d, *J* 8.6 Hz, H-4′′) and ppm 6.58 (1H, d, *J* 5.6 Hz, H-3′); δ_C (600 MHz, CD_3OD): 151.80 (C-3′′), 150.05 (C-2′), 149.46.10 (C-4), 133.60 (C-6′′), 127.53 (C-7′), 126.37 (C-8′), 124.33 (C- 5′), 124.22 (C-6′), 117.58 (C-5′′), 114.54 (C-4′′) and ppm 100.24 (C-3).

7-Chloro-4-(1,2-diaminopropyl)quinoline **13**

Off-white crystals; δ_H (600 MHz, CD_3OD): 8.34 (1H, d, *J* 5.6 Hz, H-2′), 8.12 (1H, d, *J* 9.0 Hz, H-5′), 7.76 (1H, d, *J* 2.0 Hz, H-8′), 7.39 (1H, dd, *J* 2.0 and ppm 9.0 Hz, H-6′), 6.55 (1H, d, *J* 5.6 Hz, H-3′), 3.27 (2H, dd, *J* 5.8 and 7.9 Hz, H-4′′) and ppm 3.25–3.21 (1H, m, H-3′′), 1.19 (3H, d, *J* 5.7 Hz, H-6′′); δ_C (600 MHz, CD_3OD): 150.62 (C-2′), 148.68 (C-4), 135.21 (C-7′), 128.51 (C-8′), 125.86 (C-5′), 124.38 (C-6), 99.45 (C-3′), 51.77 (C-3′′), 46.49 (C-4′′) and ppm 21.09 (C-6′′).

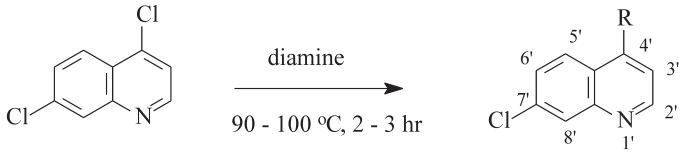
7-Chloro-4-(piperazin-1-yl) quinoline **14**

Off white crystals; δ_H (600 MHz, CD_3OD): 8.62 (1H, d, *J* 5.2 Hz, H-2′), 8.04 (1H, d, *J* 9.0 Hz, H-5′), 7.91 (1H, d, *J* 2.0 Hz, H-8′), 7.50 (1H, dd, *J* 2.0 and 9.0 Hz, H-6′), 6.99 (1H, d, *J* 5.2 Hz, H-3′), 3.24–3.20 (4H, m, H-3′′) and ppm 3.12–3.08 (4H, m, H-4′′); δ_C (600 MHz, CD_3OD): 156.88 (C-2′), 152.18 (C-4′), 133.46 (C-7′), 128.03 (C-8′), 126.10 (C-5′), 125.58 (C-6′), 109.22 (C-3′), 54.03 (C-3′′) and ppm 46.40 (C-4′′).

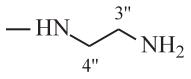
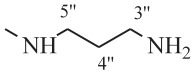
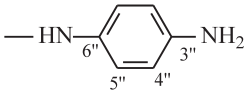
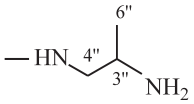
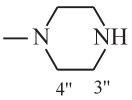
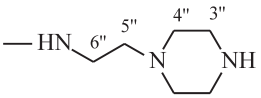
7-Chloro-4-(2-piperazinyl-ethylamino)quinoline **15**

Brown white crystals; δ_H (600 MHz, CD_3OD): 8.61 (1H, d, *J* 5.2 Hz, H-2′), 8.01 (1H, d, *J* 9.0 Hz, H-5′), 7.90 (1H, d, *J* 2.0 Hz, H-8′), 7.48 (1H, dd, *J* 2.1 and 9.0 Hz, H-6′), 6.98 (1H, d, *J* 5.2 Hz, H-3′), 3.27 (4H, s, H-4′′), 2.81 (2H, t, *J* 6.5 Hz, H-5′′), 2.76 (4H, s, H-3′′) and ppm 2.57 (2H, t, *J* 6.5 Hz, H-6′′); δ_C (600 MHz, CD_3OD): 151.96 (C-2′), 150.00 (C-4′), 133.41 (C-7′), 127.53 (C-8′), 124.14 (C-5′), 123.91 (C-6′), 98.73 (C-3), 61.30 (C-3′′), 54.16 (C-4′′), 53.08 C-5′′) and ppm 39.01 (C-6′′).

In the second step, DHA **1** is reacted with bromoethanol in the presence of boron trifluoride etherate yielding artemisinin derivative **9** (Scheme 3). Thus, to a solution of dihydroartemisinin (10.0 g, 352 mmol) and 2-bromoethanol (9.3 g, 74 mmol) in CH_2Cl_2 (40 mL), BF_3 Et_2O (15 drops) was added at



10 - 15

Compound	R
10	
11	
12	
13	
14	
15	

Scheme 2
Synthesis of amino functionalized quinoline intermediates **10**–**15**.

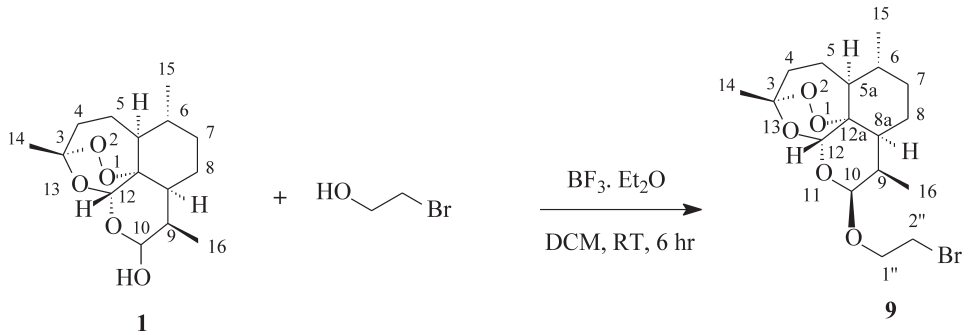
0 °C. The reaction mixture was stirred at room temperature for 6 h. After the reaction mixture was washed with a saturated $NaHCO_3$ solution, water and brine, the organic layer was dried with $MgSO_4$ and evaporated to dryness under reduced pressure. The residue was recrystallized form petroleum ether to give 9,7 g (71 % yield); of **9** as white crystals[20].

10β-(2-Bromoethoxy)-dihydroartemisinin **9**

R_f = 0.75 (DCM). δ_H (600 MHz, $CDCl_3$): 5.46 (1H, s, H-12), 4.82 (1H, d, *J* 3.4 Hz, H-10), 4.09 (1H, δ, *J* 5.5, 6.6 and 11.8 Hz, H-1''a), 3.79–3.73 (1H, m, H-1''b), 3.51–3.47 (2H, m, H-2''), 2.66–2.59 (1H, m, H-8a), 2.39–2.30 (1H, dd, *J* 3.4 and 6.4 H-9), 2.01 (1H, m, H-5a), 1.92–1.81 (m, 2H, H-4), 1.75 (2H, m, H-8), 1.73-1.66 (2H, m, H-7), 1.63-1.48 (1H, m, H-6), 1.41 (3H, s, H-14), 0.93 (3H, d, *J* 6.4 Hz, H-16) and ppm 0.91 (3H, d, *J* 7.4 Hz, H-15); δ_c (600 MHz, $CDCl_3$): 104.10 (C-3), 102.02 (C-10), 88.12 (C-12), 81.07 (C-12a), 68.14 (C-1''), 52.54 (C-5a), 44.33 (C-8a), 37.36 (C-6), 36.37 (C-4), 34.63 (C-7), 31.41 (C-2''), 30.86 (C-9), 26.12 (C-14), 24.62 (C-5), 24.33 (C-8), 20.34 (C-15) and ppm 12.95 (C-16).

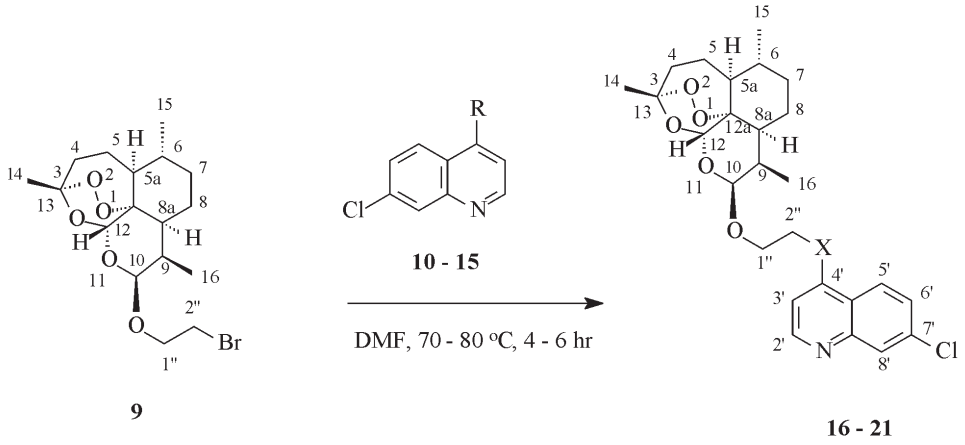
This recation usually leads to a racemate (mixture of α and β epimers). However, in this study only the 10β-epimer **9** was isolated[20].

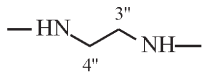
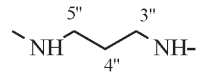
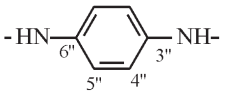
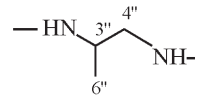
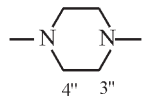
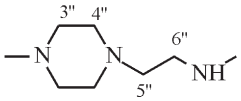
Finally, the hybrids **16**–**21** were obtained by treatment of the intermediate **9** with the various quinoline based primary/secondary amines (Scheme 4). For solubility reason, portion of each free base hybrid was converted into its oxalate salt upon equimolar reaction between the base and oxalic acid. The reaction is described as follows: an excess of dry diethylether and



1 **9**

Scheme 3
Synthesis of 2-(10β-dihydroartemisinoxy) ethylbromide 9.



Compound	X
16	
17	
18	
19	
20	
21	

Scheme 4
Synthesis of DHA-quinoline hybrids **16–21** (spacers are directed from DHA to quinoline ring).

a minimum of methanol were added to 1 equivalent of free base. Oxalic acid (1 equivalent), dissolved in dry diethylether was added dropwise to the base solution. The precipitate which formed subsequently upon stirring at room temperature for 20 mn, was filtered off and dried in vacuo to give the oxalate salts.

As the ^{1}H NMR data for the oxalate salts were a repetition to that of the free bases **16–21**, only the latters are reported. In the MS spectra of compounds **16–21,** the presence of one chlorine atom can be deduced by the presence of two peaks in a 3:1 ratio separated by 2 mass units.

{2-[(7-Chloroquinolin-4-yl)amino]ethyl}(2-{(10β-dihydroartemisinin-10-yl)oxy}ethyl)amine **16**

Hybrid **16** was obtained as yellowish oil in 59 % yield; R_f = 0.46 (DCM/MeOH 9:1). δ_H (600 MHz, $CDCl_3$): 8.44 (1H, d, *J* 5.3 Hz, H-2′), 7.88 (1H, d, *J* 1.6 Hz, H-5′), 7.69 (1H, d, *J* 8.9 Hz, H-8′), 7.28 (1H, dd, *J* 1.6 and 8.9 Hz, H-6′), 6.32 (1H, d, *J* 5.3 Hz, H-3′), 5.33 (1H, s, H-12), 4.76 (1H, d, *J* 3.3 Hz, H-10), 3.92 (1H, dt, *J* 5.0 and 10.1 Hz, H-1′′a), 3.57–3.45 (m, 1H, H-1′′b), 3.32–3.23 (2H, t, *J* 5.6 Hz, H-4′′), 3.00 (2H, t, *J* 5.6 Hz, H-3′′), 2.87–2.75 (2H, m, H-2′′), 2.58 (1H, dt, *J* 3.3 and 7.7 Hz, H-9), 1.38 (3H, s, H-14), 0.82 (3H, d, *J* 7.4 Hz, H-16) and ppm 0.80 (3H, d, *J* 5.5 Hz, H-15); δ_C (600 MHz, $CDCl_3$): 151.79 (C-2′), 149.87 (C-4′), 148.83 (C-8′), 134.74 (C-7′), 128.34 (C-8a), 125.17 (C-6′), 121.39 (C-5′), 117.24 (C-4′), 104.05 (C-3), 102.00 (C-10), 99.01 (C-3′), 87.77 (C-12), 80.84 (C-12a), 67.58 (C-1′′), 52.30 (C-5a), 48.55 (C-2′′), 47.11 (C-3′′), 43.90 (C-8a), 42.00 (C-4′′), 37.37 (C-6), 36.23 (C-4), 34.36 (C-7), 30.71 (C-9), 26.05 (C-14), 24.48 (C-5), 24.42 (C-8), 20.15 (C-15) and ppm 12.92 (C-16); $C_{28}H_{38}ClN_3O_5$ (M_w 532.07, calc.); APCI *m/z*: 532.3 (M^+, 100 %).

{3-[(7-Chloroquinolin-4-yl)amino]propyl}(2-{(10β-dihydroartemisinin-10-yl)oxy}ethyl)amine **17**

Hybrid **17** was obtained as yellow oil in 35 % yield; R_f = 0.49

(DCM/MeOH 9:1); δ_H (600 MHz, $CDCl_3$): 8.44 (1H, d, *J* 5.4 Hz, H-2′), 7.91 (1H, d, *J* 1.8 Hz, H-5′), 7.70 (1H, d, *J* 8.9 Hz, H-8′), 7.37 (1H, dd, *J* 2.0 and 8.9 Hz, H-6′), 6.28 (1H, d, *J* 5.5 Hz, H-3′), 5.38 (1H, s, H-12), 4.84 (1H, d, *J* 3.4 Hz, H-10), 4.03 (1H, dt, *J* 5.2 and 10.4 Hz, H-1a″), 3.60 (1H, dt, *J* 5.1 and 10.3 Hz, H-1b″), 3.38 (2H, t, *J* 5.9 Hz, H-5″), 2.94 (2H, t, *J* 5.4 Hz, H-3″), 2.87 (2H, t, *J* 5.2 Hz, H-2″), 2.68–2.61 (1H, m, H-9), 1.91 (2H, dt, *J* 5.6 and 11.2 Hz, H-4″), 1.42 (3H, s, H-14) and ppm 0.87 (6H, dd, *J* 6.6 and 9.4 Hz, H-15 and H-16); δ_C (600 MHz, $CDCl_3$): 151.41 (C-2′), 150.73 (C-4′), 148.40 (C-8a′), 135.00 (C-7′), 127.95 (C-8′), 125.25 (C-6′), 121.95 (C-5′), 117.38 (C-4′), 104.22 (C-3), 102.33 (C-10), 98.14 (C-3′), 87.82 (C-12), 80.90 (C-12a), 67.79 (C-1″), 52.41 (C-5a), 49.19 (C-2″ and C-3″), 44.21 (C-8a), 43.88 (C-5″), 37.46 (C-6), 36.32 (C-4), 34.46 (C-7), 30.79 (C-9), 27.11 (C-4″), 26.11 (C-14), 24.62 (C-5), 24.57 (C-8), 20.27 (C-15) and ppm 13.02 (C-16); $C_{29}H_{40}ClN_3O_5$ (M_w 546.01, calc.); APCI *m/z*: 546.34 (M^+, 100 %), 548.33.

1-N-(7-Chloroquinolin-4-yl)-4-N-(2-{(10β-dihydroartemisinin-10-yl)oxy}ethyl)benzene-1,4-amine **18**

Hybrid **18** was isolated as brownish oil in 35 % yield; R_f = 0.57 (DCM/MeOH 9:1); δ_H (600 MHz, $CDCl_3$): 8.40 (1H, d, *J* 5.4 Hz, H-2′), 7.94 (1H, d, *J* 2.1 Hz, H-5′), 7.85 (1H, d, *J* 8.9 Hz, H-8′), 7.36 (1H, dd, *J* 2.1 and 8.9 Hz, H-6′), 7.08 (2H, d, *J* 8.6 Hz, H-4″), 6.64 (2H, d, *J* 8.7 Hz, H-5″), 6.57 (1H, d, *J* 5.4 Hz, H-3′), 5.36 (1H, s, H-12), 4.81 (1H, d, *J* 3.4 Hz, H-10), 4.01 (1H, δ, *J* 4.1, 6.2 and 10.3 Hz, H-1a″), 3.64 (1H, δ, *J* 4.1, 6.5 and 10.4 Hz, H-1b″), 3.38–3.25 (2H, m, H-2″), 2.67–2.59 (1H, m, H-9), 2.34 (1H, td, *J* 3.9 and 14.1 Hz, H-4a), 2.04–1.96 (1H, m, H-4b), 1.41 (3H, s, H-14), 0.92 (3H, d, *J* 7.5 Hz, H-16) and ppm 0.91 (3H, d, *J* 1.8 Hz, H-15); δ_C (600 MHz, $CDCl_3$): 151.71 (C-2′), 149.65 (C-8′), 149.32 (C-4′), 146.45 (C-4′), 135.02 (C-7′), 128.67 (C-7″), 128.57 (C-8′), 126.64 (C-4″), 125.51 (C-6′), 121.24 (C-5′), 117.33 (C-4), 113.82 (C-5″), 104.14 (C-3), 102.19 (C-10), 101.13 (C-3′), 87.87 (C-12), 80.92 (C-12a), 66.95 (C-1″), 52.38 (C-5a), 44.20 (C-8a), 43.85 (C-2″), 37.38 (C-6), 36.30 (C-4), 34.49 (C-7), 30.78 (C-9), 26.09 (C-14), 24.60 (C-5), 24.53 (C-8), 20.27 (C-15) and ppm 13.00 (C-16); $C_{36}H_{38}ClN_3O_5$ (M_w 580.11, calc.); APCI *m/z*: 580.36 (M^+, 100 %), 582.37.

{1-[(7-Chloroquinolin-4-yl)amino]propan-2-yl}(2-{(10β-dihydroartemisinin-10-yl)oxy}ethyl)amine **19**

Compound **19** was racemate (mixture 3′′R and 3′′S isomers) isolated as fluffy light brown crystals in 45 % yield; Mp: 67 °C; δ_H (600 MHz, $CDCl_3$): 8.47 (2H, d, *J* 4.7 Hz, H-2′), 7.93–7.88 (m, 2H, H-5′), 7.71–7.66 (2H, m, H-8′), 7.33 (2H, dd, *J* 2.1 and 8.9 Hz, H-6′), 6.41–6.38 (1H, m, H-3′), 6.35–6.31 (1H, m, H-3′), 5.99 (s, 1H, H-6″), 5.75 (dd, *J* = 36.6, 5.9 Hz, 1H, H-6″), 5.37–5.33 (1H, m, H-12), 5.29 (1H, s, H-12), 4.78 (1H, dd, *J* 4.1 and 7.4 Hz, H-10), 4.76 (1H, d, *J* 3.4 Hz, H-10), 3.96 (1H, δ, *J* 3.8, 7.1 and 10.3 Hz, H-1′′a), 3.90 (1H, δ, *J* 4.3, 6.4 and 10.5 Hz, H-1′′a), 3.78 (1H, dt, *J* 5.8 and 11.8 Hz, H-3″), 3.73 (1H, dt, *J* 5.6 and 11.6 Hz, H-4″), 3.57–3.51 (1H, m, H-1′′b), 3.48 (1H, δ, *J* 3.8, 6.6 and 10.2 Hz, H-1′′b), 3.30 (1H, t, *J* 15.4 Hz, H-4′′a), 3.10 (1H, tt, *J* 6.4 and 12.9 Hz, H-3″), 3.05–2.96 (1H, m, H-4′′b), 2.96–2.75 (4H, m, H-2″), 1.40 (s, 3H, H-14), 1.38 (s, 3H, H-14), 1.27 (3H, dd, *J* 4.0 and 6.3 Hz, H-6″), 1.20 (1H, dd, *J* 3.5 and 6.3 Hz, H- 6″), 0.84–0.80 (6H, m, H-16) and ppm 0.79 (6H, dd, *J* 2.8 and 6.8 Hz, H-15); δ_c (600 MHz, $CDCl_3$): 151.90 (C-2′), 151.85 (C-2′), 149.99 (C-4′), 149.22 (C-4′), 149.15 (C-8′a), 148.93 (C-8′), 134.82 (C-7′), 128.58 (C-8′), 128.51 (C-8′), 125.32 (C-6′), 125.24 (C-6′), 121.32 (C-5′), 121.25 (C-5′), 117.49 (C-4′a), 117.34 (C-4′a), 104.11 (C-3), 104.07 (C-3), 102.04 (C-3′), 101.80 (C-3′), 99.32 (C-10), 99.11 (C-10), 87.84 (C-12), 87.75 (C-10), 80.90 (C-12a), 80.84 (C-12a), 67.80 (C-1″), 67.57 (C-1″), 54.12 (C-4″), 53.95 (C-4″), 52.33 (C-5a), 51.30 (C-5a), 49.28 (C-2″), 49.23 (C-2″), 47.59 (C-5″), 47.43 (C-5″), 44.18 (C-8a), 44.15 (C-8a), 37.43 (C-6), 37.41 (C-6), 36.29 (C-4), 36.27 (C-4), 34.40 (C-7), 30.72 (C-9), 26.11 (C-14), 24.54 (C-5), 24.50 (C-5), 24.42 (C-8), 24.39 (C-8), 20.19 (C-15), 19.18 (C-15), 18.06 (C-6′′), 17.95 (C-6′′), 12.99 (C-16) and ppm 12.90 (C-16); $C_{29}H_{40}ClN_3O_5$ (M_w 546.01, calc.); APCI *m/z*: 546.27 (M^+, 90 %), 548.29.

7-Chloro-4-[4-{2-[10β-dihydroartemisinin-10-yl]oxy}ethyl)piperazin-1-yl]quinoline **20**

Hybrid **20** was obtained as fluffy light yellow crystals in 39 % yield; Mp: 77 °C; R_f = 0.72 (DCM/MeOH 9:1); δ_H (600 MHz, $CDCl_3$): 8.69 (1H, d, *J* 4.9 Hz, H-2′), 8.01 (1H, d, *J* 1.8 Hz, H-5′), 7.91 (1H, d, *J* 8.9 Hz, H-8′), 7.39 (1H, dd, *J* 1.8 and 9.0 Hz, H-6′), 6.80 (1H, d, *J* 5.0 Hz, H-3′), 5.47 (s, 1H, H-12), 4.82 (1H, d, *J* 3.3 Hz, H-10), 4.02–3.95 (m, 1H, H-1′′a), 3.62–3.55 (m, 1H, H-1″b), 3.24–3.14 (4H, m, H-3″), 2.73 (4H, dd, *J* 6.8 and 12.5 Hz, H-4″), 2.71–2.65 (2H, m, H-2″), 2.65–2.58 (1H, m, H-9), 2.34 (1H, td, *J* 3.8 and 14.0 Hz, H-4a), 2.04–1.97 (1H, m, H-4b), 1.41 (3H, s, H-14), 0.93 (3H, d, *J* 6.4 Hz, H-16), 0.90 (3H, d, *J* 7.4 Hz, H-15); δ_c (600 MHz, $CDCl_3$): 157.00 (C-2′), 151.91 (C-4′), 134.84 (C-7′), 128.82 (C-8′), 126.09 (C-6′), 125.18 (C-5′), 108.89 (C-3′), 104.06 (C-3), 102.02 (C-10), 87.93 (C-12), 81.09 (C-12a), 65.83 (C-1″), 57.86 (C-2″), 53.28 (C-3″), 52.52 (C-5a), 52.26 (C-4″), 44.38 (C-8a), 37.54 (C-6), 36.37 (C-4), 34.65 (C-7), 30.83 (C-9), 26.17 (C-14), 24.72 (C-5), 24.40 (C-8), 20.33 (C-15) and ppm 13.07 (C-16); $C_{30}H_{40}ClN_3O_5$ (M_w 558.11, calc.); APCI *m/z*: 558.43 (M^+, 100 %), 560.25.

7-Chloro-N-{2-[4-(2-{10β-dihydroartemisinin-10-yl]oxy}ethyl)piperazin-1-yl]ethyl}quinolin-4-amine **20**

Hybrid **21** was obtained as dark yellow oil in 52 % yield; R_f = 0.51 (DCM/MeOH 9:1); δ_H (600 MHz, $CDCl_3$): 8.61 (1H, d, *J* 5.0 Hz, H-2′), 7.94 (1H, d, *J* 1.9 Hz, H-5′), 7.84 (1H, d, *J* 9.0 Hz, H-8′), 7.32 (1H, dd, *J* 2.0 and 9.0 Hz, H-6′), 6.72 (1H, d, *J* 5.0 Hz, H-3′), 5.33 (1H, s, H-12), 4.75 (1H, d, *J* 3.0 Hz, H-10), 3.90 (1H, dt, *J* 4.8 and 9.9 Hz, H-1′′a), 3.48 (1H, td, *J* 5.3 and 10.8 Hz, H-1′′b), 3.13 (4H, s, H-3″), 2.76 (2H, t, *J* 5.1 Hz, H-2″), 2.72 (2H, t, *J* 5.9 Hz, H-5″), 2.66 (4H, s, H-4″), 2.56 (3H, t, *J* 5.8 Hz, H-6″ and H-9), 2.27 (1H, td, *J* 3.8 and 14.4 Hz, H-4a), 1.94 (1H, d, *J* 14.5 Hz, H-4b), 1.34 (3H, s, H-14), 0.85 (3H, s, H-16) and ppm 0.84 (3H, s, H-15); δ_C (600 MHz, $CDCl_3$): 156.77 (C-2′), 151.70 (C-4′), 149.87 (C-8′), 134.69 (C-7′), 125.92 (C-6′), 125.06 (C-5′), 121.69 (C-4a), 108.68 (C-3′), 103.92 (C-3), 101.92 (C-10), 87.68 (C-12), 80.80 (C-12a), 67.59 (C-1″), 57.65 (C-2″), 52.96 (C-3″), 52.30 (C-5a), 52.00 (C-4″), 49.16 (C-5″), 46.05 (C-6″), 44.12 (C-8a), 37.23 (C-6), 36.19 (C-4), 34.43 (C-7), 30.67 (C-9), 25.99 (C-14), 24.48 (C-5), 24.38 (C-8), 20.17 (C-15) and ppm 13.01 (C-16); $C_{32}H_{45}ClN_4O_5$ (M_w 601.18, calc.); APCI *m/z*: 601.25 (M^+, 100 %), 603.31.

2.3. *In vitro* Antimalarial Activity

The derivatives were tested in triplicate against the D10 and Dd2 strains of *Plasmodium falciparum*. Continuous *in vitro* cultures of asexual erythrocyte stages of *P. falciparum* were maintained using a modified method reported by Trager and Jensen[21]. Quantitative assessment of antiplasmodial activity *in vitro* was determined *via* the parasite lactate dehydrogenase assay using a modified method described by Makler[22].

The test samples were prepared as a 2 mg mL^{-1} stock solution in 10 % dimethyl sulfoxide (DMSO) and sonicated to enhance solubility. Stock solutions were stored at –20 °C. Further dilutions were prepared on the day of the experiment. Chloroquine β-artemether and dihydroartemisinin were used as the reference drugs in all experiments. A full-dose response was performed for all compounds to determine the concentration inhibiting 50 % of parasite growth (IC_{50}).

For a range starting from 100 ng mL^{-1} the following was ap-

plied: The starting concentration of 100 ng mL^{-1} was serially diluted, 2-fold in complete medium to give ten dilutions with the lowest concentration being 0.2 ng mL^{-1}. Thus the concentrations considered were 100 ng mL^{-1}, 50 ng mL^{-1}, 25 ng mL^{-1}, 12.5 ng mL^{-1}, 6.25 ng mL^{-1}, 3.125 ng mL^{-1}, 1.56 ng mL^{-1}, 0.78 ng mL^{-1}, 0.39 ng mL^{-1} and 0.195 ng mL^{-1}. The same dilution technique was used for all samples. The samples are tested in triplicate. The solvents to which the parasites were exposed to had no measurable effect on the parasite viability. The ethers and the hybrids were tested in the same experimental conditions but at different dates. The IC_{50}-values were obtained using a non-linear dose-response curve fitting analysis via Graph Pad Prism v.4.0 software, and the values on molar basis in Table 2 were obtained by dividing those on mass basis by the molecular weight of each compound.

3. Results and Discussion

3.1. Chemistry

3.1.1. *Artemisinin–EG Ethers*

The new ether derivatives of artemisinin **3**–**5b** and **6a**–**8** were prepared by treatment of DHA with an appropriate MEG or EEG, respectively, in the presence of boron trifluoride diethyl etherate ($BF_3.Et_2O$) at room temperature, and were obtained with yields in the 7–51 % range. The purification was achieved by flash column chromatography on silica gel. An oxonium ion at position 11 has been suggested to be involved in the ether formation[7]. In some cases a mixture of two isomers (**5a** and **5b**, and **6a** and **6b**) was formed and they were successfully separated. The configuration at the C-10 position of the ethers was assigned based on the vicinal coupling constant $J_{H\text{-}9:H\text{-}10}$[23].

A large coupling constant between H-9 and H-10 in the case of the 10α-isomer, $J_{H\text{-}9:H\text{-}10}$ = 9–10 Hz[9], indicates the relative *trans* configuration. The 10β-isomer, on the other hand has a small coupling constant, $J_{H\text{-}9:H\text{-}10}$ = 3.6–5 Hz[8]. The relative configuration for such a compound is *cis*. Thus, compounds **3**, **4**, **5a**, **6a**, **7** and **8**, all with $J_{H\text{-}9:H\text{-}10}$ values in the 4–5 Hz range were 10β-isomers. On contrary, compounds **5b** ($J_{H\text{-}9:H\text{-}10}$ = 9.2 Hz) and **6b** ($J_{H\text{-}9:H\text{-}10}$ = 9.3 Hz) were the 10α-epimers of **5b** and **6b**, respectively (Table 1). In ^{1}H NMR spectra of these, the signals of H-12, H-10 and OCH_2 appear up field whereas those of the 10β-epimers all appear downfield[23]. Thus, in correlation with the yields, one can conclude that the experimental conditions favoured the formation of 10β-epimers in majority.

In the ^{1}H NMR spectrum of DHA, the signals of H-12 and H-10 for the 10β-epimer appeared at δ_H 5.41 (s) and 4.8 (s), respectively, whereas for the 10α-epimer they appeared at δ_H 5.33 (s) and 4.43 (s), respectively.

The ^{13}C NMR spectrum of DHA showed signals at 104.99, 99.40, 88.62, 81,42, 52.87, 45.51, 37.65, 36.84, 34.61, 32.28, 25.60, 22.99, 22.72, 20.00 and 14.87 ppm corresponding to C-10, C-3, C-12, C-12a, C-5a, C-8a, C-9, C-4, C-7, C-6, C-14, C-5, C-8, C-15 and C-16, respectively. These signals were all present in the spectra of the ethers, confirming the presence of the DHA moiety in these compounds.

The chemical structures of the title compounds **3**–**8** were confirmed by NMR and FAB-MS data. The ^{1}H NMR spectra of compounds **3**–**5b** exhibited resonances in the 3.99–3.31 ppm region characteristic of methylene hydrogen of OCH_2-CH_2O belonging to MEG chain while the signal of OCH_3 hydrogen appeared as singlet in 3.37–3.31 ppm region. The ^{1}H spectra of ethers **6a**–**8** showed a signal in 3.97–3.45 ppm region characteristic of methylene hydrogen OCH_2-CH_2O belonging to EEG chain and the presence of CH_3 of ethyl hydrogen is confirmed by the signal between 1.25–1.00 ppm. The presence of the MEG chain in the structures of **3**–**5b** was further confirmed by the resonance of the methoxy (OCH_3) carbon between 58.77 and 59.03 ppm, and OCH_2-CH_2O carbons between 71.64–67.53 ppm in ^{13}C spectra. In the structures of compounds **6a**–**8**, the presence of EEG chain was confirmed by the resonance of CH_3 of ethyl moiety between 15.08–12.40 while those of OCH_2-CH_2O resonated between 70.86–66.36 ppm in the ^{13}C NMR spectra. Furthermore, due to the close proximity to several asymmetric carbon centers on the DHA moiety, the methylene hydrogen on the carbon α adjacent to the new ether oxygen are non-equivalent and thus appear as an AB quartet[24].

Table 1 Configurational information of compounds **3**–**8** and **16**–**21**.

Compound	$J_{H\text{-}9:H\text{-}10}$ /Hz	Epimer	Configuration C-10
1		β	cis
3	4.8	β	″
4	4.5	β	″
5a	4.3	β	″
5b	9.2	α	trans
6a	4.5	β	cis
6b	9.3	α	trans
7	4.6	β	cis
8	4.4	β	″
16	3.4	β	cis
17	3.4	β	″
18	3.4	β	″
19	3.3; 4.1	β	″
20	3.3	β	″
21	3.0	β	″

3.1.2. *Artemisinin–Quinoline Hybrids*

The hybrids were obtained in yields varying from 35 to 59 % and their structure confirmed by NMR and MS spectroscopy.

Only two hybrids *viz.* **19** and **20** were solids, all others were either yellowish or brownish oils. Part of free bases hybrids **16–19** and **21** were treated with oxalic acid to obtain the oxalate salts **16a**–**19a**, and **21a**, primarily for solubility and stability reasons. DHA was supplied as a mixture of epimers, but only β-epimerr of 2-(10-dihydroartemisinoxy)ethylbromide **9** was obtained, therefore all synthesized hybrids were βepimers and were tested as such alongside the oxalate salts.

Here again, due to the close proximity to several asymmetric carbon centers on the DHA moiety, the methylene hydrogen on the carbon α adjacent to the new ether oxygen in the structure of **9**, and therefore in those of hybrids **16**–**21**, are non-equivalent and thus appear as an AB quartet [23].

Furthermore, only the relevant peaks for hybrids **16**–**21** were notated, and as the NMR data for the oxalate salts were a repetition of those of the free bases, only the latter are reported. Thus, the peaks of H-12, H-10 as well as those H-18a,b and H-19, protons of α and β methylene carbon adjacent to O-17 of DHA are noticeable in the ^{1}H spectra of all hybrids. In the MS spectra of the hybrids, the presence of one chlorine atom can be deduced by the presence of two peaks in a 3:1 ratio separated by 2 mass units.

3.2. *In vitro* Antimalarial Activity

3.2.1. *Artemisinin–EG Ethers*

The new EG oligomeric ethers of artemisinin were tested *in vitro* against both the CQS and CQR strains of *Plasmodium*

Table 2 *In vitro* antiplasmodial activity of synthesized artemisinin-PEG ethers and -quinoline hybrids

Compound	n	D10: IC_{50} /nM	S.D.	Dd2: IC_{50} /nM	S.D.	RI
3	2	0.245	0.049	0.510	0.007	2
4	3	0.045	0.002	0.039	0.001	0.9
5a	3	0.094	0.005	0.061	0.002	0.7
5b	3	0.051	0.006	0.030	0.002	0.6
6a	3	0.090	0.003	0.083	0.013	0.9
6b	3	0.051	0.007	0.025	0.002	0.5
7	3	0.051	0.005	0.032	0.004	0.6
8	3	0.030	0.003	0.023	0.001	0.8
***β*-ARM**	3	0.021	0.008	0.004	0.000	0.4
CQ [b]	3	0.060	0.004	0.473	0.013	7
16	3	0.084	0.022	0.153	0.002	1.8
16a	3	0.021	0.001	0.025	0.001	1.2
17	3	0.117	0.016	0.184	0.005	1.6
17a	3	0.014	0.003	0.020	0.001	1.4
18	3	0.030	0.003	0.069	0.002	2.3
18a	3	0.017	0.003	0.030	0.012	1.8
19 *	3	0.007	0.001	0.009	0.001	1.3
19a	3	0.008	0.001	0.011	0.002	1.4
20 *	3	0.034	0.002	0.075	0.005	2.2
21 *	3	0.218	0.022	0.300	0.076	1.4
21a	3	0.031	0.003	0.032	0.004	1
DHA	4	0.005	0.001	0.002	0.000	0.4
CQ	3	0.035	0.011	0.255	0.085	7.3

*Tested as a suspension. n = Number of data sets averaged. Resistance index (RI) = IC_{50} Dd2/IC_{50} D10. S.D. = standard deviation.

falciparum. All tested ethers were active against both the D10 and Dd2 strains. However, they were more active against the Dd2 strain, and showed comparable activity against the D10 strain as can be seen from their resistance index (RI) values in the 0.5 to 0.9 range (Table 2).

Ethers **3**, **5a** and **6a** were found less potent than ARM against either the D10 or Dd2 strain, while **5b**, **6b** and **7** showed less potency than ARM only against D10 strain. With the exception of compound **3** which was remarkably less potent than CQ against the parasites of the D10 strain [IC_{50} values; 0.245 *vs.* 0.06 nM], all other ethers showed potency similar to that of CQ against the same strain.

The activity against the CQ resistant strain revealed a picture slightly different from that against the sensitive strain. Indeed, all the ethers were active against the Dd2 strain. They were all less potent than ARM. However, compound **3** possessed potency similar to that of CQ (IC_{50} values; 0.51 nM *vs.* 0.47 nM), while all others were more potent than CQ.

In summary, compound **3** was distinctively the least active of all synthesized ethers irrespective of the strain while ether **8** was the most active. With activity comparable to that of ARM against both strains, and potency higher than that of CQ, the compound **8** lends itself as a good drug candidate to undergo pharmacokinetic studies to ascertain whether or not through that ether the half-life of artemisinin has been enhanced.

3.2.2. Artemisinin–Quinoline Hybrids

Two reference antimalarial drugs, *viz.* CQ and DHA, were tested alongside the hybrids and their salts. The results showed that compounds **16a**, **17a**, **18** and **18a**, **19** and **19a**, **20**, and **21a** displayed a good antimalarial activity profile as they had potency comparable to that of CQ against the D10 strain, and were found more active than CQ against the Dd2 strain. However, **16a**, **17a**, **19** and **19a**, and **21a** were more active against the Dd2 strain–indicated by the the resistance index RI ≤ 1.5. Hybrid **19** and its oxalate **19a** displayed the best activity profile against both strains. Compounds **16**, **17** and **21** showed poor activity against the CQS strain, which was also seen against the CQR one.

Overall the oxalates had better antiplasmodial activity than their free base hybrids, presumably due to their better solubility in the testing aqueous medium. The good activity of some of these compounds against the Dd2 strain is in agreement with the results from previous studies[25,26]. All the synthesized compounds were less active than DHA irrespective of the strain. This a major setback as one of the objectives of the study was the search for more potent antimalarials in comparison with the current artemisinins.

4. Conclusion

We have successfully synthesized series of ethylene glycol ethers and quinoline hybrids of artemisinin through derivatization at its 10-OH position. The *in vitro* antimalarial activity indicates that all the compounds were active against both D10 and Dd2 strains of *Plasmodium falciparum*. However, none of the compounds showed remarkably better activity than either artemether or dihydroartemisinin irrespective of the strain.

The majority of ethers showed higher potency than CQ against Dd2 strain. The artemisinin derivative **8** featuring 3 EO units and an ethyl side chain terminal group was the most active of all among the ethers. Hybrid **19** containing isopropyl linker on the other hand and its oxalate salt **19a** possessed the highest antimalarial activities, among the hybrids, and were indeed more potent than CQ against Dd2 strain.

Thus, ether **8,** and the hybrids **19** and **19a** lend themselves as good drug candidates to undergo further studies, namely pharmacokinetics, to ascertain whether or not the half-life of artemisinin has been increased through these compounds as one objective of this project was the search for long acting artemisinin derivatives.

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