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Research Paper

Synthesis and antimalarial activity of ethylene glycol oligomeric ethers of artemisinin

Minette Steyn[a], David D. N'Da[a], Jaco C. Breytenbach[a], Peter J. Smith[b], Sandra Meredith[b] and Wilma J. Breytenbach[c]

[a]Pharmaceutical Chemistry, [c]Statistical Consultation Services, North-West University, Potchefstroom, and [b]Pharmacology, University of Cape Town, Groote Schuur Hospital, Observatory, South Africa

Abstract

Objectives The aim of this study was to synthesize a series of ethylene glycol ether derivatives of the antimalarial drug artemisinin, determine their values for selected physicochemical properties and evaluate their antimalarial activity *in vitro* against *Plasmodium falciparum* strains.

Methods The ethers were synthesized in a one-step process by coupling ethylene glycol moieties of various chain lengths to carbon C-10 of artemisinin. The aqueous solubility and log *D* values were determined in phosphate buffered saline (pH 7.4). The derivatives were screened for antimalarial activity alongside artemether and chloroquine against chloroquine-sensitive (D10) and moderately chloroquine-resistant (Dd2) strains of *P. falciparum*.

Key findings The aqueous solubility within each series increased as the ethylene glycol chain lengthened. The IC50 values revealed that all the derivatives were active against both D10 and Dd2 strains. All were less potent than artemether irrespective of the strain. However, they proved to be more potent than chloroquine against the resistant strain. Compound **8**, featuring three ethylene oxide units, was the most active of all the synthesized ethers.

Conclusions The conjugation of dihydroartemisinin to ethylene glycol units of various chain lengths through etheral linkage led to water-soluble derivatives. The strategy did not result in an increase of antimalarial activity compared with artemether. It is nevertheless a promising approach to further investigate and synthesize water-soluble derivatives of artemisinin that may be more active than artemether by increasing the ethylene glycol chain length.

Keywords artemether; artemisinin; chloroquine; malaria; *Plasmodium falciparum*

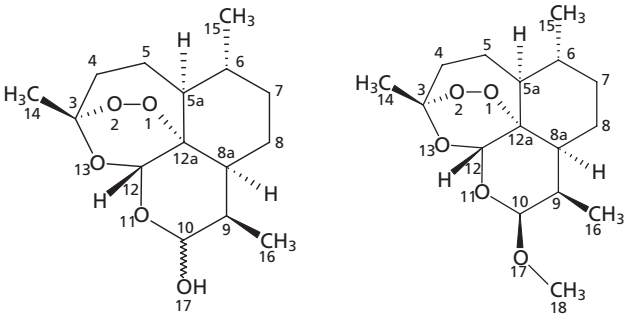
Introduction

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. The relentless increase in resistance of malaria parasites to existing and classical antimalarial drugs, such as chloroquine, sulfadoxine/pyrimethamine and mefloquine, has prompted the search for other chemotherapeutic antimalarial drugs with different molecular mechanisms of action from those to which malaria parasites have developed resistance.[1]

The artemisinin group of drugs was first discovered and developed in China. A crude extract of the wormwood plant *Artemisia annua* (qinghao) was used as an antipyretic approximately 2000 years ago, and its specific effect on malarial fever was reported in the 16th century.[2] The active constituent of the extract was identified and purified in the 1970s and was known as qinghaosu or artemisinin. Artemisinin proved effective in clinical trials in the 1980s, but a number of semi-synthetic derivatives were developed to improve the pharmacological properties and antimalarial potency of the drug.[3] Several million patients have been treated with these compounds over the past three decades owing to the increasing prevalence of multidrug resistant *Plasmodium falciparum*.[4]

Artemisinin and its derivatives showed rapid onset of action, low toxicity and high antimalarial activity against both drug-resistant and drug-sensitive malaria, in early clinical studies.[5] However, the practical value of these antimalarial agents is impaired by their poor solubility in oil and water, poor oral bioavailability, high rate of parasite recrudescence after

Correspondence: David D. N'Da, Pharmaceutical Chemistry, North-West University, Potchefstroom 2520, South Africa.
E-mail: david.nda@nwu.ac.za



1 **2**

Figure 1 Dihydroartemisinin (**1**) and β-artemether (**2**).

treatment,[6] and short plasma half-life.[7] To overcome these pharmacokinetic deficiencies, a program aimed 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 (**2**; Figure 1) and arteether,[8] and water-soluble sodium artesunate.[9] Artemether is the methyl ether derivative of artemisinin, and arteether is the ethyl ether derivative. These compounds are lipophilic and more potent than artemisinin, but still have a short plasma half-life. Artemisinin, dihydroartemisinin (**1**; Figure 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.[10] 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,[11] the high rate of recrudescence and the drug's extremely short plasma half-life.[12]

Oligomers, and especially polymers of ethylene glycols (PEG), are amphiphilic and relatively inert compounds consisting of repetitive units of ethylene oxide.[13] Pegylation, generally described as the molecular attachment of PEGs 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.[13] 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.[14] The attachment of a PEG moiety to drug molecules increases the overall size of the parent drug and the circulation half-life of PEGs increases with the increase in molecular weight.[15] Many studies indicated a dramatic enhancement in the biological half-life of particular drug molecules as a result of pegylation.[16] Pegylated drugs are also more stable over a range of pH and temperature changes[17] 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.[18]

In the search for stable, more water-soluble, highly potent, long-acting antimalarial agents, we modified the artemisinin molecule by introducing ethylene glycol moieties at the C10 position through etheral linkages. In this paper, we report the synthesis of these ethers, their physicochemical properties such as aqueous solubility and log *D*, and in-vitro antimalarial activity in comparison with the leading antimalarial drugs, artemether and chloroquine.

Materials and Methods

Materials

2-(2-Methoxyethoxy) ethan-1-ol, 2-(2-(2-methoxyethoxy) ethoxy) ethan-1-ol, 2-(2-(2-ethoxyethoxy)ethoxy) ethan-1-ol and boron trifluoride diethyl etherate ($BF_3{\cdot}Et_2O$) were all purchased from Fluka (Johannesburg, South Africa). 2-Methoxyethan-1-ol and 2-(2-ethoxyethoxy) ethan-1-ol were purchased from Acros Organics (Johannesburg, South Africa). 2-Ethoxyethan-1-ol was purchased from Sigma-Aldrich (Johannesburg, South Africa). Dihydroartemisinin (α and β racemates) was purchased from HuBei Enshi TianRanYuan Science and Technology Herbal Co., Ltd (China). HPLC grade acetonitrile was obtained from Labchem (Johannesburg, South Africa). All the reagents and chemicals were of analytical grade.

General procedures

Thin layer chromatography was performed using silica gel plates ($60F_{254}$ Merck, Johannesburg, South Africa). Preparative flash column chromatography was carried out on silica gel (230–240 mesh, G60 Merck) and silica gel 60 (70–230 mesh ASTM; Fluka). Analytical quantities of samples were weighed on a Sartorius/BP211D balance.

The ^{1}H and ^{13}C nuclear magnetic resonance (NMR) spectra were recorded on a Bruker Avance III 600 spectrometer purchased from Bruker (Karlsruhe, Germany) (at a frequency of 600.17 and 150.913 MHz, respectively) in deuterated chloroform ($CDCl_3$). Chemical shifts are reported in parts per million (δ ppm) using tetramethylsilane as internal standard. The splitting pattern abbreviations are as follows: s (singlet), d (doublet), dd (doublet of doublets), ddd (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 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 ($M+H^+$) and ($M+Na^+$) were recorded.

High performance liquid chromatography

The high performance liquid chromatography (HPLC) system purchased from Agilent Technologies (Palo Alto, CA, USA), consisted of an Agilent 1100 series auto sampler, Agilent 1100 series variable wave detector and Agilent 1100 series isocratic pump. A Zorbax Eclipse XDB C18, 5 μm (150 × 4.60 mm) column purchased from Phenomenex (Torrance, CA, USA) was used and the Agilent Chemstation rev A08.03 for LC systems software package was used for data analysis.

The compounds were quantified using a gradient method (A = 0.2% triethylamine in H_2O, pH 7.0; B = acetonitrile) at a

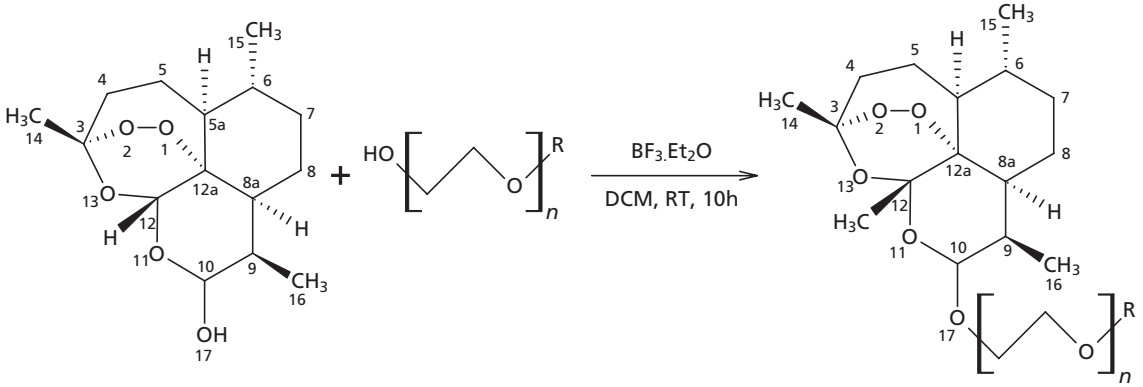
flow rate of 1 ml/min with 20-μl standard sample injections. The gradient consisted of 25% of solvent B until 1 min, then increased linearly to 95% of B after 8 min, and held for 15 min. Thereafter the instrument was re-equilibrated to the starting conditions for 5 min. A calibration plot of peak area versus drug concentration for each compound showing excellent linearity ($0.993 < r^2 \leq 1$) over the concentration range 0–2000 μg/ml was used for the assays. The absorption maximum for dihydroartemisinin and all its ether derivatives was at 205 nm; this wavelength was therefore used for the HPLC detection. The peak retention times were 10.17 min for **2**, 9.86 min for **3**, 9.78 min for **4**, 9.57 min for **5a**, 9.57 min for **5b**, 10.42 min for **6a**, 9.84 min for **6b**, 10.34 min for **7** and 10.12 min for **8**.

Synthesis of ethylene glycol oligomeric ethers of artemisinin

The synthesis of ethylene glycol ethers of artemisinin (Figure 2) was achieved by using the general method reported by Li *et al*.,[19] with slight modifications. To a solution of dihydroartemisinin (**1**) (2.0 g, 7 mmol) and methoxypoly(ethylene glycol) (MPEG) or ethoxypoly(ethylene glycol) (EPEG) (14 mmol, 2.0 eq. relative to dihydroartemisinin) dissolved in 50 ml of anhydrous dichloromethane (DCM, CH_2Cl_2), was added $BF_3{\cdot}Et_2O$ (1.0 ml) portionwise at 0°C. The mixture was stirred at 0°C for 30 min, then at room temperature for 10 h. The progress of the reaction was monitored by thin layer chromatography. 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 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.

2-Methoxyethoxy-10β-dihydroartemisinin (3) (R = CH₃, n = 1)

Ether **3** was purified by flash silica gel column chromatography eluting with DCM/EtOAc (20 : 1) to give a clear oil: 0.91 g (38%) yield. $C_{18}H_{30}O_6$. ^{1}H NMR (600 MHz, $CDCl_3$) δ (ppm): 5.41 (s, 1H, H-12), 4.80 (d, J = 4.8 Hz, 1H, H-10), 3.95–3.87 (m, 1H, O-*CH*-CH_2, H-18a), 3.58–3.46 (m, 3H, O-*CH*-*CH*$_2$, H-18b and H-19), 3.37–3.31 (m, 3H, O*CH*$_3$), 2.64–2.54 (m, 1H), 2.34 (td, J = 14.2, 3.7 Hz, 1H), 2.00 (d, J = 14.4 Hz, 1H), 1.88–1.57 (m, 5H), 1.51–1.38 (m, 4H), 1.35–1.15 (m, 3H), 0.95–0.82 (m, 6H). ^{13}C NMR (151 MHz, $CDCl_3$) δ (ppm): 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 (C5a), 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), 19.46 (C-16). MS FAB 343.3 [(M+H+) 8%], 365.4 [(M+Na$^+$) 4%].



1 **3–8**

Compound	Isomer	R	*n*
3	10β	CH_3	1
4	10β	CH_3	2
5a	10β	CH_3	3
5b	10α	CH_3	3
6a	10β	CH_2CH_3	1
6b	10α	CH_2CH_3	1
7	10β	CH_2CH_3	2
8	10β	CH_2CH_3	3

Figure 2 Synthesis of ethylene glycol oligomeric ethers of artemisinin. DCM, dichloromethane; RT, room temperature.

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

Derivative **4** was purified by flash silica column chromatography eluting with DCM/EtOAc (15 : 1) to produce a light yellow oil: 1.28 g (47%) yield. $C_{20}H_{34}O_7$. ^{1}H NMR (600 MHz, $CDCl_3$) δ (ppm): 5.41 (s, 1H, H-12), 4.80 (d, J = 2.4 Hz, 1H, H-10), 3.95–3.87 (m, 1H, O-*CH*-CH_2, H-18a), 3.58–3.46 (m, 7H, O*CH*-*CH_2*-O-*CH_2*-*CH_2*-O, H-18b-19-21-22), 3.37–3.31 (m, 3H, O*CH_3*), 2.64–2.54 (m, 1H), 2.34 (td, J = 14.2, 3.7 Hz, 1H), 2.00 (d, J = 14.4 Hz, 1H), 1.88–1.57 (m, 5H), 1.51–1.38 (m, 4H), 1.35–1.15 (m, 3H), 0.95–0.82 (m, 6H). ^{13}C NMR (151 MHz, $CDCl_3$) δ (ppm): 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), 12.87 (C-16). MS FAB 387.3 [(M+H^+) 12%], 409.3 [(M+Na^+)11%].

2-(2-(2-Methoxyethoxy)ethoxy) ethoxy-10β-dihydroartemisinin (5a) (R = CH_3, n = 3)

Compound **5a** in a yield of 1.45 g (48%) of yellowish oil was obtained after purification with flash chromatography eluting with DCM/EtOAc (10 : 1). $C_{22}H_{38}O_8$. ^{1}H NMR (600 MHz, $CDCl_3$) δ (ppm): 5.36 (s, 1H, H-12), 4.76 (d, J = 4.3 Hz, 1H, H-10), 3.91–3.82 (m, 1H, O-*CH*-CH_2, H-18a), 3.62–3.48 (m, 11H, O*CH*-*CH_2*-O-*CH_2*-*CH_2*-O-*CH_2*-*CH_2*-O, H-18b-19-21-22-24-25), 3.31 (s, 3H, O*CH_3*, H-27), 2.55 (d, J = 3.1 Hz, 1H), 2.29 (td, J = 14.1, 3.5 Hz, 1H), 1.96 (d, J = 14.4 Hz, 1H), 1.83–1.65 (m, 4H), 1.55 (d, J = 12.9 Hz, 1H), 1.43–1.34 (m, 4H), 1.29–1.11 (m, 3H), 0.86 (dd, J = 23.3, 6.8 Hz, 6H). ^{13}C NMR (151 MHz, $CDCl_3$) δ (ppm): 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), 12.87 (C-16). MS FAB 431.4 ((M+H^+) 5%), 453.4 ((M+Na^+) 25%).

2-(2-(2-Methoxyethoxy)ethoxy) ethoxy-10α-dihydroartemisinin (5b) (R = CH_3, n = 3)

Ether **5b** was purified by flash silica gel column chromatography eluting with DCM/EtOAc (10 : 1) to give a yellow oil: 0.36 g (12%) yield. $C_{22}H_{38}O8$. ^{1}H NMR (600 MHz, $CDCl_3$) δ (ppm): 5.26 (s, 1H, H-12), 4.44 (d, J = 9.2 Hz, 1H, H-10), 3.97 (t, J = 8.7 Hz, 1H, O-*CH*-*CH_2*-O, H-18a), 3.59 (dd, J = 16.2, 6.2 Hz, 9H, O-*CH*-*CH_2*-O-*CH_2*-*CH_2*-O-*CH_2*, H-18b-19-21-22-24), 3.49–3.46 (m, 2H, *CH_2*-OCH_3, H-25), 3.31 (s, 3H, *OCH_3*, H-27), 2.32 (ddd, J = 16.7, 13.5, 5.0 Hz, 2H), 1.95 (d, J = 14.4 Hz, 1H), 1.80 (d, J = 13.6 Hz, 2H), 1.68 (d, J = 13.4 Hz, 1H), 1.61 (d, J = 13.1 Hz, 1H), 1.50–1.35 (m, 5H), 1.27–1.17 (m, 3H), 0.95–0.81 (m, 6H). ^{13}C NMR (151 MHz, $CDCl_3$) δ (ppm): 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), 12.55 (C-16). MS FAB 431.1 ((M+H^+) 5%), 453.1 ((M+Na^+) 11%).

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

Compound **6a** afforded 0.86 g (35%) yield as a clear oil after been purified by flash silica gel column chromatography eluting with DCM/EtOAc (15 : 1). $C_{19}H_{32}O_6$. ^{1}H NMR (600 MHz, $CDCl_3$) δ (ppm): 5.41 (s, 1H, H-12), 4.93 (d, J = 4.5 Hz, 1H, H-10), 3.95–3.90 (m, 1H, O*CH*-CH_2-O, H-18a), 3.62–3.45 (m, 5H, O-*CH*-*CH_2*-O-*CH_2*, 18b-19-21), 2.29–2.20 (m, 1H), 1.99 (dd, J = 42.6, 25.3 Hz, 1H), 1.85–1.80 (m, 1H), 1.74–1.67 (m, 1H), 1.58–1.48 (m, 3H, O-CH_2-*CH_3*), 1.39–1.33 (m, 5H), 1.24–1.13 (m, 8H), 0.89 (dd, J = 21.7, 9.5 Hz, 4H). ^{13}C NMR (151 MHz, $CDCl_3$) δ (ppm): 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), 15.08 (C-22). MS FAB 357.4 ((M+H^+) 10%), 379.3 ((M+Na^+) 7%).

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

Derivative **6b** was purified by flash silica column chromatography eluting with DCM/EtOAc (15 : 1) to produce a clear oil: 0.17 g (7%) yield. $C_{19}H_{32}O_6$. ^{1}H NMR (600 MHz, $CDCl_3$) δ (ppm): 5.27 (s, 1H, H-12), 4.45 (d, J = 9.3 Hz, 1H, H-10), 3.97 (dd, J = 10.7, 4.3 Hz, 1H, O*CH*-CH_2-O, H-18a), 3.60–3.46 (m, 5H, O-*CH*-*CH_2*-O-*CH_2*, H-18b-19-21), 2.55 (d, J = 3.3 Hz, 1H), 2.33 (ddd, J = 28.3, 13.7, 5.7 Hz, 2H), 2.00–1.90 (m, 1H), 1.85–1.51 (m, 3H, O-CH_2-*CH_3*), 1.51–1.32 (m, 4H), 1.32–1.07 (m, 5H), 1.00–0.75 (m, 8H). ^{13}C NMR (151 MHz, $CDCl_3$) δ (ppm): 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), 12.40 (C-22). MS FAB 357.4 ((M+H^+) 10%), 379.3 ((M+Na^+) 8%).

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

A yield of 1.34 g (48%) of light yellow oil was obtained after purification with flash chromatography eluting with DCM/EtOAc (10 : 1). $C_{21}H_{36}O_7$. ^{1}H NMR (600 MHz, $CDCl_3$) δ (ppm): 5.37 (s, 1H, H-12), 4.76 (d, J = 4.6 Hz, 1H, H-10), 3.92–3.86 (m, 1H, O*CH*-CH_2-O, H-18a), 3.62–3.45 (m, 9H, O*CH*-*CH_2*-O-*CH_2*-*CH_2*-O-*CH_2*, H-18b-19-21-22-24), 2.55 (s, 1H), 2.29 (dd, J = 19.4, 8.6 Hz, 1H), 1.96 (d, J = 14.5 Hz, 1H), 1.77 (dd, J = 28.0, 11.6 Hz, 1H), 1.70–1.54 (m, 3H, O-CH_2-*CH_3*), 1.44–1.35 (m, 5H), 1.26 (d, J = 5.7 Hz, 1H), 1.16 (dt, J = 13.9, 6.0 Hz, 4H), 0.90–0.82 (m, 7H). ^{13}C NMR (151 MHz, $CDCl_3$) δ (ppm): 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), 12.97 (C-25). MS FAB 401.5 ((M+H$^+$) 8%), 423.4 ((M+Na$^+$) 7%).

2-(2-(2-ethoxyethoxy)ethoxy) ethoxy-10β-dihydroartemisinin (8) (R = CH_2-CH_3, n = 3)

Ether **8** was purified by flash silica gel column chromatography eluting with DCM/EtOAc (5 : 1) to give a yellow oil: 1.60 g (51%) yield. $C_{23}H_{40}O8$. ^{1}H NMR (600 MHz, $CDCl_3$) δ (ppm): 5.35 (s, 1H, H-12), 4.75 (d, J = 4.4 Hz, 1H, H-10), 3.90–3.83 (m, 1H, H-18a), 3.60–3.52 (m, 13H, O*CH*-*CH_2*-O-O-*CH_2*-*CH_2*-O-*CH_2*-*CH_2*-O-*CH_2,* H-18b-19-21-22-24-25-27), 3.45 (m, 1H, H-27), 2.54 (d, J = 3.4 Hz, 1H), 2.32–2.26 (m, 1H), 1.93 (dd, J = 13.4, 10.8 Hz, 1H), 1.73 (ddd, J = 41.2, 22.1, 8.8 Hz, 3H), 1.54 (d, J = 13.1 Hz, 1H), 1.48–1.35 (m, 5H, O-CH_2-*CH_3*), 1.25–1.13 (m, 6H), 0.82 (dd, J = 9.9, 7.6 Hz, 6H). ^{13}C NMR (151 MHz, $CDCl_3$) δ (ppm): 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), 12.94 (C-28). MS FAB 467 ((M+Na$^+$) 38%).

Physicochemical properties

Solubility

The aqueous solubility values (S_W) of crystalline compound **1** and the oils **3–5a** and **6a–8** were obtained in phosphate buffer at pH 7.4. Each compound was added in excess to the buffer and the slurries were stirred with magnetic bars in a water bath at 37°C for 24 h. An excess of solute/oil was present at all times to provide saturated solutions. After 24 h, the solution was filtered in case of compound **1** and analysed directly by HPLC to determine the concentration of solute dissolved in the solvent. For the oils, after 24 h stirring, the mixtures were allowed to stand at room temperature and then the aqueous layers were separated and analysed by HPLC. The experiment was performed in triplicate for each compound. The aqueous solubility of compound **5b** could not be determined because of insufficient quantity.

Experimental log D

Equal volumes of *n*-octanol and phosphate buffered solution of pH 7.4 were saturated with each other under vigorous stirring for at least 24 h. An accurately weighed amount (2 mg) of each derivative was dissolved in 0.75 ml of pre-saturated *n*-octanol and the solution was then stoppered and agitated for 10 min in 2-ml graduated tubes (0.5 ml division). Subsequently, 0.75 ml of pre-saturated buffer was transferred to the tubes containing the above mentioned solutions.

The tubes were stoppered and agitated for 45 min, then centrifuged at 1503*g* for 30 min. The *n*-octanol and aqueous phases were allowed to separate at room temperature for 5 min, and thereafter their volume ratio (v/v, *n*-octanol/buffer) was determined. The volume ratio was found in all cases to be 1. The *n*-octanol and aqueous phases were diluted with acetonitrile and analysed by HPLC. From these data the concentrations of the derivative in both phases were determined. The log *D* values were calculated as logarithmic ratios of the concentrations in the *n*-octanol phase to the concentrations in the buffer. The experiment was performed in triplicate.

In-vitro antimalarial activity

The derivatives were tested in triplicate against D10 and Dd2 strains of *P. falciparum*. Continuous in-vitro cultures of asexual erythrocyte stages of *P. falciparum* were maintained using a modified method reported by Trager and Jensen.[20] Quantitative assessment of antiplasmodial activity *in vitro* was determined by the parasite lactate dehydrogenase assay using a modified method described by Makler *et al*.[21]

The test samples were prepared as a 2 mg/ml stock solution in 10% dimethylsulfoxide and sonicated to enhance solubility. Stock solutions were stored at −20°C. Further dilutions were prepared on the day of the experiment. Chloroquine and β-artemether were used as the reference drugs in all experiments. A full dose–response analysis was performed for all compounds to determine the concentration inhibiting 50% of parasite growth (IC50). The samples were tested at a starting concentration of 1000 ng/ml, which was then serially diluted 2-fold in complete medium to give 10 concentrations, with the lowest concentration being 2 ng/ml. The same dilution technique was used for all samples. Test samples were re-tested at a starting concentration of 100 ng/ml. The solvents to which the parasites were exposed had no measurable effect on the parasite viability. The IC50 values were obtained using a non-linear dose–response curve fitting analysis using Graph Pad Prism version 4.0 software.

Statistical analysis

The following statistical procedures were used to test if there were significant differences between the mean IC50 values of the test compounds **3–8** and each of the standards, artemether and chloroquine. This was done for each of the D10 and Dd2 strains. One-way analysis of variance was used to determine if significant differences existed between the means of the test compounds and each of artemether and chloroquine in general. Normal probability plots on the residuals were done in each analysis to ensure that the data were fairly normally distributed.[22] Dunnett's test was performed to determine if the means of the test compounds differed significantly from the means of each of the standard compounds. Tukey's multiple comparison tests were performed to determine if the means of the test compounds differed significantly from one another for each of the D10 and Dd2 strains, omitting the standard compound from the analyses in order to determine which of the test compounds were more efficient than the others. These procedures were done using the Statistica data analysis software system, version 8.0.[23] All tests were done at a 0.05 significant level. In the case of *P* values slightly greater than 0.05, interpretations were made on a 0.10 level.

Results

Chemistry

The newly synthesized ethylene glycol oligomeric ethers of artemisinin **3–5a** and **6a–8** were obtained with yields over the

Table 1 Physicochemical properties of compounds 2–8

Compound	R	*n*	MW (g/mol)	Yield (%)	$J_{H\text{-}9:H\text{-}10}$ (Hz)	Isomer	S_W (mM)[a]	Log D[a]	S_{OC}[b] (mM)
2			298.38			β	0.10 ± 0.03	1.92 ± 0.18	8.32
3	Methoxy	1	342.43	38	4.8	β	1.37 ± 0.09	1.27 ± 0.24	25.51
4	Methoxy	2	386.48	47	4.5	β	1.66 ± 0.05	0.96 ± 0.20	15.14
5a	Methoxy	3	430.53	48	4.3	β	3.23 ± 0.1	0.53 ± 0.03	10.95
5b	Methoxy	–	430.53	12	9.2	α	ND	0.78 ± 0.24	ND
6a	Ethoxy	1	356.45	35	4.5	β	0.14 ± 0.03	1.42 ± 0.17	3.68
6b	Ethoxy	–	356.45	7	9.3	α	0.20 ± 0.03	1.69 ± 0.06	9.80
7	Ethoxy	2	400.51	48	4.6	β	0.52 ± 0.08	1.17 ± 0.23	7.69
8	Ethoxy	3	444.56	51	4.4	β	2.00 ± 0.09	0.80 ± 0.02	12.62

$J_{H\text{-}9:H\text{-}10}$, coupling constant between H-10 and H-9; log D, partition coefficient (*n*-octanol/PBS, pH 7.4); MW, molecular weight; *n*, ethylene oxide repeating unit; ND, not determined; R, substituent; S_{OC}, solubility in octanol; S_w, aqueous solubility. [a]Determined experimentally; each value represents the mean ± SD of three measurements. [b]Calculated from log S_{OC} = log D + log S_W.

range of 7–51%, and their structures were confirmed by NMR and MS spectroscopy.

Aqueous solubility and experimental log D

The compounds, irrespective of the series (methoxy or ethoxy) were all more water-soluble than artemether. The aqueous solubility increased within each series as the chain lengthened. The comparison between series revealed the methoxy series **6a–8** to be more hydrolipophilic than the ethoxy series **3–5a**. The average results are reported in Table 1.

In-vitro antimalarial activity

All compounds irrespective of the series were active against both strains of *P. falciparum*. All the ethers were found to be less potent than artemether irrespective of the strain. However, they were more potent than chloroquine against the resistant strain. The in-vitro antiplasmodial activities are reported as IC50 values in Table 2.

Discussion

Chemistry

The new ether derivatives of artemisinin were prepared by treatment of dihydroartemisinin with an appropriate methoxypoly(ethylene glycol) (**3–5b**) or ethoxypoly(ethylene glycol) (**6a–8**) in the presence of $BF_3.Et_2O$ at room temperature. 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.[11] 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 $J_{H\text{-}9:H\text{-}10}$.[24]

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,[12] 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.[11] 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 over 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-isomers of **5b** and **6b**, respectively. In ^{1}H NMR spectra of these, the signals due to H-12, H-10 and OCH_2 appear upfield, whereas in the 10β-isomers, they all appear downfield.[24] Thus, in correlation with the yields, one can conclude that the experimental conditions favoured the formation of 10β-isomers.

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

The ^{13}C NMR spectrum of dihydroartemisinin 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 dihydroartemisinin 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 the MPEG chain, while the signal of OCH_3 hydrogen appeared as a singlet in the 3.37–3.31 ppm region. The ^{1}H spectra of ethers **6a–8** showed a signal in the 3.97–3.45 ppm region, characteristic of methylene hydrogen of OCH_2-CH_2O belonging to the EPEG chain, and the presence of CH_3 of ethyl hydrogen was confirmed by the signal between 1.25 and 1.00 ppm. The presence of the MPEG 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 and 67.53 ppm in ^{13}C spectra. In the structures of compounds **6a–8**, the presence of the EPEG chain was confirmed by the resonance of CH_3 of the ethyl moiety between 15.08 and 12.40, while those of OCH_2-CH_2O resonated between 70.86 and 66.36 ppm in the ^{13}C NMR spectra. Furthermore, due to the close proximity to several asymmetric carbon centres on the dihydroartemisinin moiety, the methylene hydrogen on the carbon α adjacent to the new ether oxygen are non-equivalent and thus appear as an AB quartet.[25]

The FAB-MS data for the compounds confirmed the presence of molecular ions (m/z) at 343.3 (M+H+) and 365.4

Table 2 Descriptive statistics of IC50 values, results of analysis of variance and Dunnett's tests, and antimalarial activity of ethylene glycol oligomeric ethers of artemisinin (**3–8**), artemether and chloroquine

Compound	D10						Dd2						RI
	n	Mean IC50 (nM)	SD	*P* value: analysis of variance	*P* value: Dunnett's test		*n*	Mean IC50 (nM)	SD	*P* value: analysis of variance	*P* value: Dunnett's test		
					Artemether	Chloroquine					Artemether	Chloroquine	
3	2	0.245	0.049		0.000*	0.000*	3	0.510	0.007		0.000*	0.000*	2.1
4	3	0.045	0.002		0.146	0.679	3	0.039	0.001		0.000*	0.000*	0.9
5a	3	0.094	0.005		0.000*	0.053**	3	0.061	0.002		0.000*	0.000*	0.6
5b	3	0.051	0.006		0.054**	0.944	3	0.030	0.002		0.000*	0.000*	0.6
6a	3	0.090	0.003		0.000*	0.100**	3	0.083	0.013		0.000*	0.000*	0.9
6b	3	0.051	0.007		0.051**	0.953	3	0.025	0.002		0.001*	0.000*	0.5
7	3	0.051	0.005		0.051**	0.953	3	0.032	0.004		0.000*	0.000*	0.6
8	3	0.030	0.003		0.883	0.110	3	0.023	0.001		0.002*	0.000*	0.8
β-Artemether	3	0.021	0.008	0.00*			3	0.004	0.000	0.00*			0.5
Chloroquine	3	0.060	0.004	0.00*			3	0.473	0.013	0.00*			7.9

D10, chloroquine-sensitive strains of *Plasmodium falciparum*; Dd2, moderately chloroquine-resistant strains of *P. falciparum*; *n*, replicates; RI, resistance index (IC50 Dd2/IC50 D10). Chloroquine was tested as diphosphate salt. *Statistically significant at $P < 0.05$ compared with artemether and chloroquine. **Statistically significant at $P < 0.01$ compared with artemether and chloroquine.

(M+Na$^+$) for **3**, at 387.3 (M+H$^+$) and 409.3 (M+Na$^+$) for **4**, at 431.4 (M+H$^+$) and 453.4 (M+Na$^+$) for **5a**, at 431.1 (M+H$^+$) and 453.1 (M+Na$^+$) for **5b**, 357.4 (M+H$^+$) and 379.3 (M+Na$^+$) for **6a**, at 357.4 (M+H$^+$) and 379.3 (M+Na$^+$) for **6b**, at 401.5 (M+H$^+$) and 423.4 (M+Na$^+$) for **7**, at 467.0 (M+Na$^+$) for **8**, corresponding to the molecular formulae $C_{18}H_{30}O_6$, MW = 342.43 (**3**), $C_{20}H_{34}O_7$, MW = 386.48 (**4**), $C_{22}H_{38}O_8$, MW = 430.53 (**5a** and **5b**), $C_{19}H_{32}O_6$, MW = 356.45 (**6a** and **6b**), $C_{21}H_{36}O_7$, MW = 400.51 (**7**) and $C_{23}H_{40}O_8$, MW = 444.56 (**8**). These formulae in turn indicate the number of ethylene oxide units, *n*, in the series (R = CH_3) to be 1 for **3**, 2 for **4** and 3 for **5a** and **5b**, as well as the number of ethylene oxide units, *n*, in the series (R = CH_2-CH_3) to be 1 for **6a** and **6b**, 2 for **7** and 3 for **8**.

Aqueous solubility and experimental log D

Aqueous solubility and lipophilicity influence the way a drug molecule passes through biological membranes and barriers to ultimately enter the systemic circulation. A drug molecule must possess some lipophilic properties to permeate biological membranes and hydrophilic properties to be taken up in the systemic circulation. Thus, optimal solubility to both water and octanol is a prerequisite for drug intended to be administered orally. The logarithm of the ratio of octanol solubility to water solubility (log P), is thus found in almost every physicochemical analysis for reportedly being related to drug absorption.[26,27]

Oligomers and polymers of ethylene glycols exhibit the ability to increase the hydrophilicity of molecules with the increasing number of ethylene oxide units in the chain as a result of increased H-bond formation between water molecules and the intrachain oxygen atoms of ethylene oxide.[28] As the chain length increases so does the number of ethylene units, which ultimately increases lipid solubility.[29] The results of this study corroborate these facts. Indeed, irrespective of the series (ethoxy or methoxy), the aqueous solubility increased as the chain lengthened. However, the methoxy series proved to be more hydrophilic than the ethoxy one. Thus, for a given *n* value, the methoxy derivative showed greater water solubility than its ethoxy counterpart. When one considers $n = 1$ for example, compound **3** is more water soluble than both **6a** and **6b** (1.37 *versus* 0.14 or 0.2 mM). All synthesized ethers were found to be more water soluble than *β*-artemether (S_w = 0.1 mM). The distribution coefficient (log D), a pH dependent version of the partition coefficient (log P), was determined experimentally, and the lipid solubility of all compounds was evaluated by calculating the solubility in octanol (S_{OC}) from the experimental S_W and log D data using the equation: $\log S_{OC} = \log D + \log S_W$. The log *D* values were determined by HPLC in an *n*-octanol/buffer (pH 7.4) mixture.[29] This value of pH is supposed to reflect the cytosolic pH in the parasite.[30]

The results given in Table 1 revealed that all compounds were more lipophilic than octanol itself (all S_{OC} values were greater than 1), but the trends with increasing ethylene oxide chain length were different in the two series. The lipid solubility decreased as the chain lengthened in the methoxy series, whereas, unexpectedly, it increased in the ethoxy series. Since the ethyl moiety is more lipophilic than the methyl, one would have expected the opposite of the present trend. No plausible

explanation could be found to justify these results. Overall, the lipid solubility appeared greater for the methoxy series than the ethoxy one, except for compound **5a** *versus* compound **8**. All methoxy derivatives were more lipophilic than β-artemether. Moreover, for a given *n* value, where both 10α and 10β anomers were isolated, the α-isomer seemed to be more water and lipid soluble than its β counterpart as can be seen from the data of compound **6b** ($S_w = 0.2$ mM, $S_{OC} = 9.8$ mM) compared with **6a** ($S_w = 0.14$ mM, $S_{OC} = 3.68$ mM).

On the basis of S_W and S_{OC} values, compounds **7**, **6b**, **5a** and **8**, which were as lipid soluble as β-artemether (**2**) but possess 5-, 2-, 32- and 20-fold higher water solubility, respectively, could be absorbed faster than β-artemether, and therefore displayed greater antimalarial activity. On the contrary, compound **3**, the most hydrophobic of all, could experience slower absorption as already reported by Bigucci *et al*.[31] This is because an increase in hydrophilicity does not necessarily increase the ability of a molecule to be absorbed since the principal physicochemical properties (H-bonding properties, molecular and shape, polarity, flexibility, charge/ionization) as a whole, rather than just hydrophobicity, are better predictors of absorption.[31]

In-vitro antimalarial activity

The new ethylene glycol oligomeric ethers of artemisinin were tested *in vitro* against both chloroquine-sensitive and chloroquine-resistant strains of *P. falciparum*. All tested ethers were active against both D10 and Dd2 strains. However, they were more active against the resistant strain than against the sensitive one, with resistance index values below 1.

Ethers **3**, **5a** and **6a** were found to be less potent than artemether at a $P < 0.05$ level, while **5b**, **6b** and **7** showed less potency than artemether at a $P < 0.1$ level against D10 strain.

The IC50 of 0.245 nM compound **3**, higher than that of chloroquine (0.06 nM), differed significantly from chloroquine at a $P < 0.05$ level, and was also found to be less potent than chloroquine against D10. Ethers **5a** and **6a**, on the other hand, were significantly less potent than chloroquine at a $P < 0.10$ level against the same strain, as indicated by their mean IC50 values which were higher than that of chloroquine.

Against the resistant strain Dd2, all the compounds differed significantly from artemether at a $P < 0.05$ level. With mean IC50 values higher than that of artemether, they were less potent than artmether. All compounds (**3–8**) differed significantly ($P < 0.05$) from chloroquine against Dd2. Compound **3** was significantly less potent than chloroquine given its mean IC50 was higher than that of chloroquine (0.51 nM *versus* 0.47 nM). Ethers **4–8**, on the other hand, were significantly more potent than chloroquine, their IC50 values being lower than that of chloroquine.

Compound **3** was clearly the least active of all compounds irrespective of the strain as it had the highest IC50. Derivatives **4**, **5b**, **6b**, **7** and **8** were the most active, and showed comparable potency against the D10 strain.

Compounds **8**, **6b** and **5b** had comparable activity and were the most potent against Dd2. Derivative **4** was as potent as **7**, and both were less potent than **8** against the chloroquine-resistant strain. Ether **5a** was more potent than its ethyl counterpart **6a** against that same strain. Furthermore, irrespective of the strain, where both α- and β-isomers where tested, the α-isomer was distinctly more potent than its β counterpart as can be seen from the IC50 values of **5b** and **5a** (0.03 nM *versus* 0.061 nM), and **6b** and **6a** (0.025 *versus* 0.083 nM).

Overall, no structure–activity relationship could be established from this study. Compound **8**, the most active ether possessed aqueous solubility comparable with that of ether **3**, the least active of all the compounds, but had 2 times lower solubility in octanol than **3**. This suggests that a balance between hydrophilic and lipophilic properties is necessary for the enhancement of the antimalarial activity of ethylene glycol linked ether derivatives of artemisinin.

Conclusions

We synthesized a series of ethylene glycol oligomeric ethers of artemisinin through derivatization at the 10-OH position. The compounds were characterized by NMR and MS spectroscopy. Their log *D* values and aqueous solubility were determined experimentally. The aqueous solubility increased with increasing chain length within the series. The in-vitro IC50 data indicated that all compounds were active against both chloroquine-sensitive (D10) and chloroquine-resistant (Dd2) strains of *P. falciparum*. None of the ethers showed greater potency than artemether irrespective of the strain. However, the majority showed greater potency than chloroquine against the resistant strain. The compound featuring three ethylene oxide units and an ethoxy as side chain terminal moiety was the most active of all the derivatives, and is a good candidate for further investigation in the search for an alternative to chloroquine.

Declarations

Conflict of interest

The Author(s) declare(s) that they have no conflicts of interest to disclose.

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