Antiplasmodial Activity and Cytotoxicity of 10β-Aminoquinolinylethylethers of Artemisinin

Abstract

Background: Each year roughly 800,000 people die of malaria, with 95% being African children. The shortcomings of the current drugs and the emergence of *P. falciparum* resistance to the artemisinin class of compounds warrant the search for new classes or derivatives. In search for such compounds, a series of 10β-aminoquinolinylethylethers of artemisinin, previously synthesized from this laboratory were screened for antimalarial activity against both the chloroquine-susceptible 3D7 and -resistant K1 strains of *P. falciparum*. Their cytotoxicity was also assessed against HEK 293 and HepG2 cell lines.

Methods: The parasitic and mammalian cells were incubated with compounds at various concentrations for 72 h. The antimalarial activity was determined using SYBR Green I-based fluorescence. For cytotoxicity determination, cells were grown to confluence and CellTiter-Glo luminescent cell viability assay was used.

Results: All derivatives proved to be active against both strains with good selectivity towards the parasitic cells. The derivative 11 featuring 2 artemisinin moieties and an aminoethylpiperazine linker was the most active of all. It possessed 17- and 166-fold more potency than artemether against 3D7 (EC₅₀: 9.5 vs. 166 nM) and K1 (10.9 vs. 1723.3 nM), respectively, while was found to be as potent as artesunate against both strains.

Conclusion: Derivative 11 stands as a good candidate to be further investigated primarily in vitro in comparison with an equimolar combination of chloroquine (CQ) and artemisinin to ascertain its advantages, if any, over the combination.

Introduction

It is estimated that malaria kills about 655,000 people each year, 91% of whom are living in Africa and, most of them children under the age of 5 years [1]. The development and spread of multidrug resistant (MDR) *P. falciparum* (*P. falciparum*) has led to the adoption of artemisinin-based combination therapies (ACTs) as the first-line treatment for *falciparum* malaria in most malaria-endemic countries of the world [2]. However, the recently confirmed emergence of artemisinin resistance in western Cambodia is a major threat for current initiatives to control and eliminate malaria [3–5]. While artemisinin resistance has not yet spread to other areas [6], the World Health Organization (WHO) is coordinating a large-scale elimination campaign in this region aiming to contain the spread of resistance [7,8]. The ACTs combine fast-acting artemisinin (ART) derived drugs with other antimalarials possessing longer half-lives such as mefloquine. Because the utility of artemisinin is limited by its solubility in both oil and water, this sesquiterpene has been structurally modified by derivatization into short-chain oil soluble ether derivatives of dihyd roartemisinin (DHA, 2), such as artemether (AM, 2a) and arteether (AE, 2b), and the water soluble sodium artesunate (AS, 2c) (Fig. 1). Thus, all derivatives currently in use are either alkyl acetals or an ester acetal derivative of dihyd roartemisinin (DHA, 2). The problem with these semi-synthetic compounds, however, is their short pharmacological half-lives, a reflection of their acid lability, and facile metabolism to DHA [9–12]. In particular, artesunate is hydrolytically unstable, even at neutral pH, and has a half-life of only a few minutes [13]. A large amount of work has been carried out with the aim of generating new derivatives [14–16]. Artemisone (Fig. 2) is thus far the only second-generation semi-synthetic artemisinin derivative.
that has been found suitable for further clinical development, progressing through Phase II but showing no major benefits over existing derivatives [17]. A trioxaquine (PA1103/SAR116242) based on hybrid strategy with a dual mode of action proved to be highly active in vitro on several sensitive and resistant strains of *P. falciparum* at nanomolar concentrations and also on multidrug-resistant strains obtained from isolates. It has been selected as an antimalarial drug candidate for development [18]. Artesunate-resistant strains obtained from isolates. It has been selected of to be highly active existing derivatives [17]. A trioxaquine (PA1103/SAR116242) on *P. falciparum* previously synthesized 10

We herein report the antimalarial activity and cytotoxicity of pre-

and more recently 10-aminoethylethers [27] , hybrids and hybrid dimers with aminoquinoline [25 , 26] . Their modula tion reaction between both 10β-bromoethyl ether artemisinin and amino-functionalyzed quinoline intermediates led to the target 10β-aminooxazoline)ethylethers of artemisinin in yields varying from 15 to 59% (Step 3). They were all obtained as the 10-β-isomers and had the cis configuration at carbon C-10 of the artemisinin moiety. For stability and solubility reasons, the free base target compounds were converted into oxalate salts and were tested as such for in vitro antimalarial activity as well as cytotoxicity. The detailed synthesis and characterization of these compounds are reported elsewhere [25,26]. Their structures are delineated in the Fig. 3.

**Methods**

10β-(Aminooxazoline)ethylethers of artemisinin 3–11

The aminooxazolineylethers of the present study were previously synthesized by our group in a 3-step process involving dihydroartemisinin, 4,7-dichloroquinoline and various amines, and briefly described as follows: The condensation of DHA 2 with 2-bromoethanol in the presence of boron trifluoride etherate (BF₃, Et₂O) yielded the 10β-bromoethyl ether artemisinin inter-

mediate (step 1). In parallel reactions, the quinoline ring was amino-functionalized at its position 4 by bimolecular nucleophile substitutions involving 4,7-dichloroquine and various diamines to afford 4-amino-functionalized quinoline intermediates (step 2). Another bimolecular nucleophilic substitu-

tion reaction between both 10β-bromoethyl ether artemisinin and amino-functionalized quinoline intermediates led to the target 10β-(aminooxazoline)ethylethers of artemisinin in yields varying from 15 to 59% (Step 3). They were all obtained as the 10-β-isomers and had the cis configuration at carbon C-10 of the artemisinin moiety. For stability and solubility reasons, the free base target compounds were converted into oxalate salts and were tested as such for in vitro antimalarial activity as well as cytotoxicity. The detailed synthesis and characterization of these compounds are reported elsewhere [25,26]. Their structures are delineated in the Fig. 3.

**Methodology for in vitro biological evaluation**

Determination of antimalarial effective concentration (EC₅₀)

The artemisinin derivatives 3–11 were screened against the chloroquine-sensitive (CQS, 3D7) and chloroquine-resistant (CQR, K1) strains of *P. falciparum*. Continuous in vitro cultures of sexual erythrocyte stages of *P. falciparum* were maintained using a modified method of Trager and Jensen [28]. Asynchronous parasites were grown in the presence of fresh group O-positive erythrocytes (Lifeblood, Memphis, TN) in Petri dishes at a hematocrit of 4–6% in RPMI-based medium consisting of RPMI 1640 supplemented with 0.5% AlbuMAX II, 25 mM HEPES, 25 mM NaHCO₃ (pH 7.3), 100 μg/mL hypoxanthine, and 5 μg/mL gentamicin. Cultures were incubated at 37°C in a gas mixture of 90% N₂, 5% O₂, and 5% CO₂. For EC₅₀ determinations, 20 μL of RPMI 1640 with 5 μg/mL gentamicin were dispensed per well in an assay plate (384-well microtiter plate, clear-bottom, tissue-

treated). Next, 40 μL of each compound, previously serial diluted in a separate 384-well white polypropylene plate, were dis-

pensed in the assay plate, and then 20 μL of a synchronized cul-

ture suspension (1% rings, 10% hematocrit) were added per well to make a final hematocrit and parasitemia of 5% and 1%, respec-

tively. Assay plates were incubated for 72 h, and the parasitemia was determined by a method previously described [29]. Briefly, 10 μL of 10X Sybr Green I, 0.5% v/v Triton, and 0.5 mg/mL saponin

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**Fig. 1** Structures of artemisinin 1 and its clinically used derivatives; dihydroartemisinin 2, artemether 2a, arteether 2b and sodium artesunate 2c.

**Fig. 2** Structures of the semi-synthetic artemisinin derivative artemisone and the synthetic ozonides OZ277 and OZ439.

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solution in RPMI were added per well. Assay plates were shaken for 30 s, incubated in the dark for 4 h, and then read with the Envision spectrofluorometer at Ex/Em 485 nm/535 nm. EC50s were calculated using proprietary software developed in house [30] in the Pipeline Pilot environment that pools data from all replicates of the experiment and fits a consensus model. The values reported in the Table are the results of a statistical analysis performed by pooling the data of 6 replicates (2 runs and 3 replicates per run) and not the arithmetic means of the 2 independent runs.

**In vitro cytotoxicity against HEK293 and HEP G2 cells**

HEK293 and HepG2 cell lines were purchased from the American Type Culture Collection (ATCC, Manassas, VA) and were cultured according to recommendations. Cell culture media were purchased from ATCC. HEK 293 (human embryonic kidney) and HepG2 (human liver hepatocellular carcinoma) are both mammalian cell lines. Exponentially growing cells were plated in Corning 384 well white custom assay plates, and incubated overnight at 37 °C in a humidified, 5% CO2 incubator. DMSO inhibitor stock solutions, the same as in the antimalarial assay, were added the following day to a top final concentration of 25μM, 0.25% DMSO and then diluted 1/3 for a total of 10 testing concentrations. Cytotoxicity was determined following a 72-h incubation using Promega Cell Titer Glo Reagent according to the manufacturer’s recommendation. Luminescence was measured on an Envision plate reader (Perkin Elmer). EC50s were calculated using proprietary software as in the antimalarial assay [30].

**Dose-response curve fitting**

Dose-response curves were calculated from percent activity values and log10-transformed concentrations the proprietary robust interpretation of screening experiments (RISE) application written in Pipeline Pilot (Accelrys, v. 8.5) and the R program [30]. A non-linear regression was performed using the R drc package with the 4-parameter log-logistic function (LL2.4) [31].

**Results**

The in vitro biological evaluation of the compounds was performed at the Department of Chemical Biology and Therapeutics, St. Jude Children’s Research Hospital, Memphis, Tennessee, US. 10β-(Aminoquinolinyl)ethylethers of artesiminin 3–11, also artemisinin-quinoline hybrids 3–9 and artemisinin-quinoline hybrids dimers 10–11, were screened for antimalarial activity.
against 3D7 and K1, 2 strains of *P. falciparum*. 3D7 and K1 are CQS and CQR strains, respectively. Also described alongside them, were the clinically used derivatives, artemether 2a and sodium artesunate 2c. The results of both antiparasomal activity and cytotoxicity are reported in the Table 1.

Furthermore, antiparasomal activity and cytotoxicity, expressed as inhibitory concentration (IC₅₀) against D10 and Dd2 strains of *P. falciparum*, and CHO cells, respectively, previously determined at the Department of Pharmacology, University of Cape Town, South Africa [25, 26], are also compiled in the Table for comparison sake.

As can be seen, all tested derivatives were active against both strains with good selectivity towards the parasitic cells, even better than AM and AS. Hybrid 8 was the least whereas dimer 11 was the most active of all. Derivative 8 showed activity comparable to that of artemether against 3D7 while all compounds were found to possess activities 7–17 times higher. Against the K1 strain, hybrid 8 was still the least active, however, being 4 times more potent than AM. All others were significantly (>10-times) more potent, dimer 11 displaying an outstanding 160-times higher activity than AM.

In comparison to ARS, the derivatives 3–10 proved to be less potent against both strains while 11 was found as potent as artesunate against both strains.

Furthermore, derivatives 3–7 and 9–11 have RI (resistance index) values ~1 indicating that these compounds retained their activity even against the CQR K1 strain. On the contrary, derivative 8 and artemether possess RI values of 2.4 and 10.4, respectively.

### Discussion

The *in vitro* antiparasomal activity of compounds 3–11 have already been evaluated against the D10 (CQS) and Dd2 (CQR) strains of *P. falciparum*. In that early work, all compounds were active against both strains. The oxalate salt of compound 5 stood as the most active of all. This compound was found to be less potent than 2 but displayed a significant 7 times higher potency than CQ against Dd2 [26].

### Conclusion

A series of 9 10β-(aminoquinolinyl)ethylether derivatives of artemisinin of proven in vitro antimalarial activity against CQS D10 and CQR Dd2, were screened against another 2 strains of *P. falciparum* viz. CQS 3D7 and CQR K1. They were all also active against both latter strains with good selectivity towards the parasitic cells. However, the dimeric compound 11 featuring 2 artemisinin moieties and an aminoethylpiperazine linker was...
distinctively the most active of all. It displayed an outstanding potency in the nanomolar range over arteether and was found equipotent to artesunate against both strains. This compound lends itself as a good candidate to be further investigated to ascertain whether this excellent in vitro activity can be carried over in vivo. It will be interesting to assess through pharmacokinetic study if this compound possess prolonged half-life in comparison to the parent drug artemisinin or its clinical derivatives, and whether it operates as artemisinin or/and chloroquine.

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

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