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 were assessed against HEK 293 and HepG2 cell lines.

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 dihydroartemisinin (DHA, 2), such as arteether (AE, 2b) and artesunate (AS, 2c) (Fig. 1). Thus, all derivatives currently in use are either alkyl acetals or an ester acetal derivative of dihydroartemisinin (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]. Arte-lanef (Fig. 1, OZ277) has a longer half-life, is fast acting, well tolerated, and effective. It was in Phase III clinical trials in combination with piperaquine [20,21], and its maleate salt is now registered in India as Synriam, a drug combination product with piperaquine phosphate [22]. OZ439 (Fig. 2), a synthetic ozonide, is a drug candidate designed to provide a single-dose oral cure in humans. It has successfully completed Phase I clinical trials, where it was shown to be safe at doses up to 1.6 g, and is currently undergoing Phase IIa trials in malaria patients [23]. On the basis of their structural differences, it is believed that these synthetic ozonides may replace the standard artemisins if they become obsolete [17]. Nevertheless, the shortcomings of the current drugs, the emergence of *P. falciparum* resistance to the artemisinin class of compounds in South East Asia, and the fact that the mechanism by which the parasite acquires resistance is still largely unknown, warrant the search for new classes or derivatives.

As part of our contribution to these efforts, we embarked on an on-going program focusing on the design, synthesis and antimalarial activity evaluation of new antimalarials. The structural modification of the artemisinin molecule to generate new derivatives is at the core of this program. We have already reported the synthesis and antiplasmodial activity of several artemisinin derivatives including oligomeric ethers with ethylene glycol [24], hybrids and hybrid dimers with aminoquinoline [25,26] and more recently 10-aminoethylethers [27].

We herein report the antimalarial activity and cytotoxicity of previously synthesized 10β-aminoquinolinylethylethers of artemisinin to highlight their inhibitory potential against a variety of *P. falciparum* strains.

![Fig. 1 Structures of artemisinin 1 and its clinically used derivatives; dihydroartemisinin 2, artemether 2a, arteether 2b and sodium artesunate 2c.](image1)

**Methods**

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

The aminoquinolinylethers 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 (BF$_3$, Et$_2$O) yielded the 10β-bromoethylether artemisinin intermediate (step 1). In parallel reactions, the quinoline ring was amino-functionalized at its position 4 by bimolecular nucleophilic substitutions involving 4,7-dichloroquinoline and various diamines to afford 4-amino-functionalized quinoline intermediates (step 2). Another bimolecular nucleophilic substitution reaction between both 10β-bromoethylether artemisinin and amino-functionalized quinoline intermediates led to the targeted 10β-(aminoquinolinyl)ethylethers of artemisinin in yields varying from 15 to 59% (Step 3). They were all obtained as the 10β-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 (EC$_{50}$)

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 asexual 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 NaHCO$_3$ (pH 7.3), 100 μg/mL hypoxanthine, and 5 μg/mL gentamicin. Cultures were incubated at 37 °C in a gas mixture of 90% N$_2$, 5% O$_2$, and 5% CO$_2$. For EC$_{50}$ 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 nL of each compound, previously serial diluted in a separate 384-well white polypropylene plate, were dispensed in the assay plate, and then 20 μL of a synchronized culture suspension (1% rings, 10% hematocrit) were added per well to make a final hematocrit and parasitemia of 5% and 1%, respectively. 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

![Fig. 2 Structures of the semi-synthetic artemisinin derivative artemisone and the synthetic ozonides OZ277 and OZ439.](image2)
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 antiplasmodial assay, were added the following day to a top final concentration of 25 μM, 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 antiplasmodial 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 artemisinin 3–11, also artemisinin-quinoline hybrids 3–9 and artemisinin-quinoline hybrids dimers 10–11, were screened for antimalarial activity.

![Fig. 3 Structures of 10β-(aminoquinolinyl)ethylethers of artemisinin 3–11 obtained from DHA 2, 4,7-dichloroquinoline and various diamines in a 3-step process [25,26].](image)
Table 1  In vitro antimalarial activity of 10β-aminooquinolinylethylethers of artesinin 3–11 against 3D7 and K1 strains of P. falciparum, and their cytotoxicity against HEK293 and HepG2 cell lines.

| Comp. | Activity EC50 (nM)a | Activity IC50 (nM)b | Cytotoxicity EC50 (µM)c | Cytotoxicity IC50 (µM)d | SI 1 | SI 2 | HEK293 | HepG2 | CHO | SI 1 | g | SI 2 | h | SI i |
|-------|-------------------|-------------------|---------------------|---------------------|-----|-----|--------|--------|-----|-----|-----|-----|-----|-----|-----|
|       | 3D7   | K1   | D10  | Dd2 | RIa | RIb | HEK293 | HepG2  | CHO | D10 | 26 | Dd2 | 26 | 26 |
| 3     | 41.6  | 44.2 | 21.5 | 25.7| 1.1 | 1  | >24.4 | >24.4 | 1.7 | >587 | >587 | >1119 | >1119 | >2039 |
| 4     | 52.7  | 54.9 | 14.3 | 19.8| 1.0 | 1  | 9.8   | 4.6   | 0.2 | 186  | 87  | 12  |
| 5     | 35.5  | 34.1 | 14.9 | 20.8| 1.0 | 1  | 14.0  | 16.1  | 3.4 | 394  | 454 | 184 |
| 6     | 85.6  | 99.7 | nd   | nd  | 1.2 | 1  | >24.6 | >24.6 | >287| >287 |
| 7     | 63.4  | 68.2 | nd   | nd  | 1.1 | 1  | >23.3 | >23.3 | >368| >368 |
| 8     | 199.4 | 479.3| 29.0 | 29.2| 2.4 | 1  | >26.1 | >26.1 | 2.3 | >130 | >130 | 80  |
| 9     | 23.5  | 22.2 | 17.3 | 30.2| 1.0 | 2  | >26.3 | >26.3 | 35.2| >1119| >1119| 2039|
| 10    | 36.6  | 33.8 | 5.3  | 28.4| 0.9 | 5  | >22.0 | >22.0 | 0.7 | >601 | >601 | 128 |
| 11    | 9.5   | 10.9 | 19.6 | 55.7| 1.2 | 3  | >23.0 | >23.0 | 74.8| >2421| >2421| 3813|
| AM 2a | 166   | 1723.3| 10.4 | 26.0| >0.2| >0.2|
| AS 2c | 6.6   | 6.6  | 1    | >26.0| >3.9| >3.9|


Discipline

The in vitro antimalosomal 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].

In the current study, compounds 3–11 proved to be active against both strains as well. The EC50 values varied in the 10–90 nM range. The dimeric compound 11 displayed the most effective activity and was found to be as potent as artesunate against both 3D7 and K1, though, it had previously been reported as less potent than that reference drug against the Dd2 strain. The comparison of the activity of 11 against the CQS (D10 vs. 3D7) and CQR (Dd2 vs. K1) strains reveals a meaningful difference. It was found to be slightly more active against 3D7 than D10, and also a 5 times more active against K1 than Dd2.

Compound 5, the most active compound in the previous study carried over its performance in the present one. There was no meaningful difference between activity against D10 and Dd7, and against Dd2 and K1. When the comparison was extended to the remaining compounds viz. 3–4 and 6–10, it clearly appeared that these were more active against D10 than Dd7, and more active against Dd2 than K1 strain. Thus, the artemisinin derivatives 3–11 were overall more active against D10 than Dd7, and Dd2 than K1. This suggests that although both D10 and Dd7 are considered to be CQ sensitive, these strains of different cell lines have different behaviors in the presence of the compounds. Similarly, Dd2 and K1 are considered to be CQ resistant, and yet these artemisinin derivatives have different behaviors against them.

Furthermore, the fact that hybrid 8 and artemether possess RI values higher than 1 may suggest that perhaps the K1 strain is on the verge of developing resistance against these 2 compounds. Thus, through this study, the 10β-aminooquinolinylethylethers of artemisinin 3–11 were confirmed to be active in vitro against 4 different strains of P. falciparum.

Conclusion

A series of 9 10β-aminooquinolinylethylether 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 aminoethylpyrazine linker was...
distinctively the most active of all. It displayed an outstanding potency in the nanomolar range over artemether 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/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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