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Research paper

Synthesis and antimalarial evaluation of artesunate-polyamine and trioxolane-polyamine conjugates

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ABSTRACT

A series of artesunate-polyamine and trioxolane-polyamine conjugates have been prepared. The conjugates were evaluated for antimalarial activity towards the K1 dual drug resistant and NF54 chloroquine-sensitive strains of *Plasmodium falciparum* (*Pf*) and for cytotoxicity towards the rat myoblast cell line L6. (Bis)-Boc-(bis)-artesunate-polyamine and (tetra)-artesunate-polyamine conjugates exhibited potent in vitro activity towards both strains of *Pf*, with IC_{50} values in the range of 0.3–1.1 nM, comparable to the parent artesunate. Cytotoxicity within this series of analogues typically increased with polyamine (PA) chain length, identifying the PA3-4-3 (spermine), and to some extent the PA3-7-3 series, as being highly selective towards the parasite. The corresponding series of (bis)-Boc-(bis)-trioxolane and (tetra)-trioxolane-polyamine conjugates were less active as antimalarials than the parent trioxolane acid, highlighting the limitation of using this warhead for drug-conjugate studies. Preliminary in vivo evaluation of two artesunate-polyamine conjugates **11** and **16** demonstrated 95.5–99.8% reduction in parasitaemia with maximal 30 day survival rates (ip delivery). Oral testing of **11** proved less efficacious, with 95.7% activity and inconsistent survival rates of 16–30 days. In contrast, trioxolane-polyamines were substantially less effective (ip delivery), exhibiting only modest reductions in parasitaemia and modest to no increase in survival rates.

1. Introduction

The impact of artemisinin (**1**) (Fig. 1), a sesquiterpene trioxolane isolated from the sweet wormwood plant (*Artemisia annua*) on the treatment of malaria has been extraordinary [1,2]. With effectiveness against both chloroquine-sensitive and chloroquine-resistant malaria and a rapid onset of activity, artemisinin and semi-synthetic derivatives including artesunate (**2**) are increasingly being used for the treatment of malaria. The initially reported potent activity towards *Plasmodium falciparum* was tempered somewhat by the natural product's lipophilic nature and short half-life [1]. However, when combined with longer lived quinoline-based antimalarials, an artemisinin drug combination can rapidly clear parasites leading to a cure. Such artemisinin combination therapy (ACT) is now the recommended first-line treatment for uncomplicated *P. falciparum* malaria. In the search for new antimalarials related to the artemisinins, simple 1,2,4-trioxolanes are being developed [3], with current interest focused on arterolane (**3**), which in phase 3 trials as a fixed dose combination with piperaquine phosphate showed comparable efficacy and safety to the artemether-lumefantrine drug combination [4], and artefenomel (**4**) which exhibits single-dose cures of uncomplicated malaria in animal studies [5–7].

Single pill or multi-pill combinations are currently used to treat malaria infections. An interesting avenue of research in the search for treatments of neglected diseases is the development of multi-target-directed ligands (MTDLs) [8], whereby drugs with different mechanism of action are combined into a new chemical entity. Such a drug hybrid would potentially overcome issues associated with patient compliance, treatment completion and slow the onset of drug resistance. Previous reports of antimalarial hybrids include an artemisinin-quinine hybrid which was more potent than either drug alone or in a non-covalent mixture [9], and artemisinin-primaquine hybrids which displayed enhanced in vitro and in vivo activities [10].

As part of our ongoing search for new leads in antimalarial

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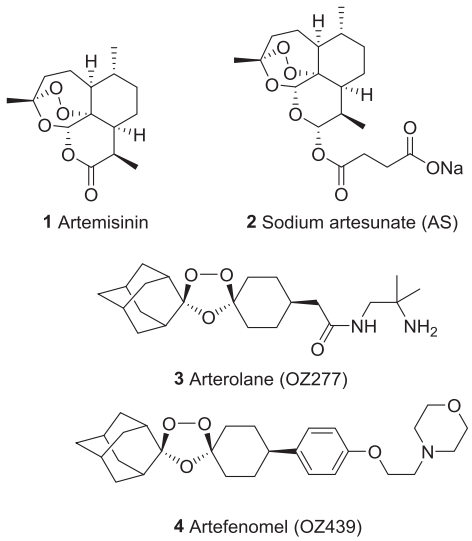


Fig. 1. Artemisinin **1**, sodium artesunate **2**, and trioxolane antimalarials arterolane **3** and artefenomel **4**.

treatment [11a-g], we recently reported structure-antimalarial relationship studies of 1,ω-diamido- and 1,ω-diglyoxylamido-polyamines related to the marine natural product orthidine F (**5**) (Fig. 2) [11f,12,13]. Antimalarial potency and cytotoxic selectivity was observed to vary with changes in structure of the 'capping acids' (phenylacetic acid, indole-3-glyoxylic acid) and the length and degree of substitution of the polyamine 'core'.

Polyamines (PAs) are ubiquitous naturally-occurring amines which play multifunctional roles including regulating cell growth and differentiation, exhibiting both promoting and protecting activities in apoptosis [14–16], modulating cell signalling pathways [17,18], and interacting with transmembrane ion channels [14,17]. Polyamine analogues have been reported to act as epigenetic modulators [19–21], and to exhibit antiproliferative [22,23], antiparasitic [24–29] and antibacterial [30–32] activities. Beyond de novo biosynthesis, many cell types, including tumour cells and malaria parasites [33], utilise polyamine transport systems to take up polyamines from exogenous sources [34,35]. Polyamine conjugates thus offer the potential to target specific cell types that upregulate polyamine transporters, acting as a vector/carrier for drug delivery [36,37].

There are very few reports of the synthesis and biological evaluation of artemisinin-alkylamine conjugates in the literature. In two studies, N-alkyl and amide-linked artemisinin-spermidine conjugates were found to exhibit variable levels of antiplasmodial activity and cytotoxicity, with Boc-protected analogues showing more potent activity [38], while amongst a series of amino-ethylether derivatives of artemisinin, shorter chain compounds were more active as antimalarials than longer chain variants [39]. More recently, homodimers of artesunate [40] linked via 1,3-diaminopropane, 1,3-diamino-2-propanol, *meta*-xylylenediamine and 1,8-diaminooctane [41,42] were reported to exhibit moderate cytotoxicity towards the CCRF-CEM and CEM/ADR5000 leukemia cell lines. Motivated by these results, we sought to prepare novel artesunate-polyamine conjugates that explored polyamine length and degree of substitution and to evaluate all analogues for their antimalarial and cytotoxic properties. For comparison, we also prepared a corresponding series of 1,2,4-trioxolane-polyamine conjugates and evaluated them in the same biological assays.

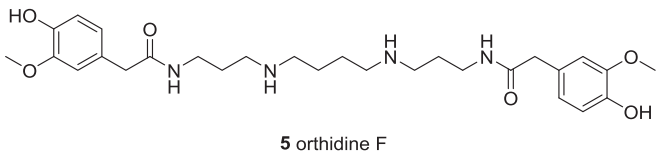


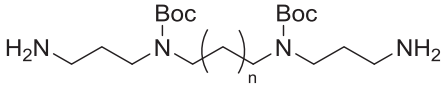
Fig. 2. Marine natural product orthidine F **5**.

2. Chemistry

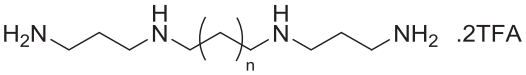
Of the targeted Boc-protected polyamine (**6a**-**6e**) and unprotected polyamine (**7a**-**7e**) scaffolds (Fig. 3), previously syntheses have been reported for all but the Boc-protected PA3-10-3 compound (**6e**). The general pathway utilised for the syntheses of **6a**-**6e** and **7a**-**7e** has been reported previously [13,30,43,44] – the synthesis of **6e**/**7e** is shown in Scheme 1, and all other polyamine scaffolds were synthesized in the same manner. 1,10-Diaminodecane **8** was (bis)cyanoethylated using acrylonitrile in ethanol to give **9**, which was then protected using Boc_2O/Et_3N in MeOH to afford (bis)-N-Boc protected intermediate **10**. Compound **10** was reduced using 10% Pd/C with 50% Ni-Al alloy under H_2 at 50 °C to give (bis)-*N*-boc protected polyamine **6e**. Compound **6e** was then used to prepare artesunate or ozonide derivatives as detailed below, or was deprotected using TFA in CH_2Cl_2 to afford the previously described polyamine PA3-10-3 (**7e**).

The target artesunate-polyamine conjugates were then prepared by EDCl-HOBt-mediated coupling of artesunate with either Boc-polyamines **6a**–**6e**, to afford Boc-protected diamides **11**–**15** in yields of 40–97%, or with polyamines **7a**–**7e** to give tetra-artesunate analogues **16**–**20** in yields of 43–71%.

We sought to compare the antimalarial activities of the artesunate-polyamine conjugates with a corresponding library of synthetic ozonide-polyamine conjugate analogues. The potently antimalarial 1,2,4-trioxolane warhead **21** (Fig. 4) was prepared via a literature route [45], and subsequent coupling with Boc-polyamines **6a**-**6e** using CDI gave conjugates **22**–**26** in yields of 37–94%, while coupling with polyamines **7a**-**7e**, using HATU and DIPEA in DMF gave tetra-ozonide substituted conjugates **27**–**31** in yields of 24–64% (Scheme 2).



6a n = 2
6b n = 4
6c n = 5
6d n = 6
6e n = 8



7a n = 2
7b n = 4
7c n = 5
7d n = 6
7e n = 8

Fig. 3. Target polyamine scaffolds **6**-**e** and **7a**-**e**.