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# Optimisation of the synthesis of second generation 1,2,4,5 tetraoxane antimalarials

Paul M. O' Neill [a,*], Sunil Sabbani [a], Gemma L. Nixon [a], Matthew Schnaderbeck [b], Natalie L. Roberts [a], Emma R. Shore [b], Christopher Riley [a], Ben Murphy [a], Paul McGillan [a], Stephen A. Ward [c], Jill Davies [c], Richard K. Amewu [d]

[a] Department of Chemistry, University of Liverpool, Liverpool, L69 7ZD, UK
[b] Eisai Inc. 4 Corporate Dr, Andover, MA 01810, USA
[c] Research Centre for Drugs and Diagnostics, Liverpool School of Tropical Medicine, Pembroke Place, Liverpool, L3 5QA, UK
[d] Department of Chemistry, University of Ghana, P. O. Box LG56, Legon, Accra, Ghana

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ABSTRACT

An efficient route to the synthesis of potent antimalarial aryloxy 1,2,4,5-tetraoxanes is described that permits parallel synthesis for Structure–Activity Relationship (SAR) investigations. Brief details of the in vitro and in vivo antimalarial evaluation are included which enables identification of antimalarial leads for further development. Also described is an improved approach to the synthesis of a selected late-lead compound in just four or five synthetic steps from commercially available starting materials.



## 1. Introduction

Artemisinin (**1a**) and its semi-synthetic derivatives **1b** and **1c** remain at the forefront of antimalarial chemotherapy due to their high potencies, safety and their ability to clear malaria parasites more rapidly than any other clinically used class (Fig. 1).[1–3] In spite of these properties, first generation compounds have short plasma half lives that result in malaria parasite recrudescence.[1] This has led to extensive medicinal chemistry efforts to find fully synthetic endoperoxide alternatives with improved potency and pharmacokinetic characteristics. In addition to very short plasma half-lives, there are now reports of resistance to the artemisinins in South East Asia and it is hoped that novel fully synthetic, structurally distinct, longer half-life endoperoxides can circumvent parasite resistance.[4]

Currently, the most advanced synthetic peroxide drug developed is OZ277 (**2c**), an endoperoxide with outstanding antimalarial activity and improved pharmacokinetic characteristics when compared with the artemisinins. OZ277 was approved in 2012 and is used in a drug-combination with piperaquine (trade name; Synriam).[5] The structure of this drug contains a 1,2,4-trioxolane core and a spiroadamantane unit. The 1,2,4 trioxolane group mimics the endoperoxide bridge found in artemisinin and mechanistic studies have shown that if the peroxide bond is too exposed, as in structure **2a**, the peroxide bond becomes susceptible to cleavage, and therefore expresses no antimalarial activity. Functionalisation of the endoperoxide group with a spiroadamantane group on one side, results in a compound **2b**, that expresses high antimalarial potency. This SAR observation is thought to be as a result of accessibility of the peroxide bridge to Fe(II) via an energetically favourable approach, whilst the O–O linkage still has steric protection from the bulky adamantane caged ring system (Fig. 1).[6]

Over time, further medicinal chemistry optimisation of the 1,2,4-trioxolane core was performed to provide a second generation compound, OZ439 (**3a**). Aryloxy substituted 1,2,4-trioxolane OZ439 demonstrates increased drug exposure and half-life when compared to the alkyl substituted OZ277 with an improvement in antimalarial properties.[7] A full review of the OZ439 trials can be found in the report by Charman et al. and Phase II clinical trials are currently underway.[8]

Reliance on semi-synthetic artemisinin derivatives with a single synthetic peroxide back-up class limits the ability to respond to the malaria elimination challenge in terms of ensuring we have options

* Corresponding author. E-mail address: pmoneill@liv.ac.uk (P.M. O' Neill).

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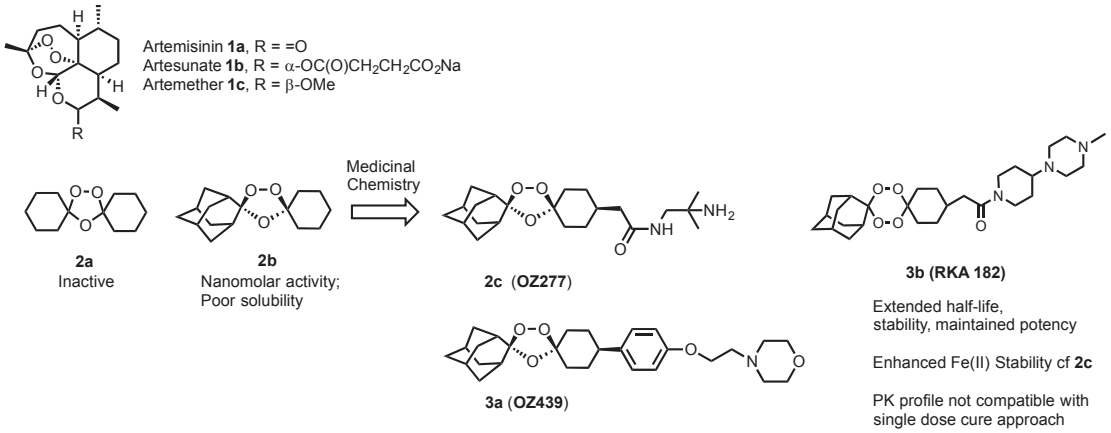


**Fig. 1.** Structure of artemisinin (**1a**), semi-synthetic analogues artesunate (**1b**), artemether (**1c**) and synthetic peroxides under development.

in the face of potential resistance and the need to generate new drug combinations with a range of pharmacological and chemical characteristics going forward. Following the early seminal work of Vennerstrom on tetraoxane antimalarials,[10] we initiated studies on the development of orally active tetraoxane molecules and produced an optimized lead compound RKA182 (**3b**) with in vitro antimalarial activity in the single digit nanomolar range versus multi-drug resistant isolates, improved rodent pharmacokinetic (PK) profiles and oral activity versus the artemisinins in the mouse model of malaria.[10] However, the PK of this molecule is not compatible for its potential use within a single dose cure regimen, the new benchmark for developmental antimalarial drugs. As a result, additional optimisation was deemed necessary to extend the stability, PK and in vivo performance of this molecule.[11]

## 2. Results and discussion

Based upon the beneficial effects seen by incorporation of an aryl ring into the side-chain of the OZ series, we decided to investigate the synthesis and SAR of a head to head series of 1,2,4,5-tetraoxanes. For this programme we decided to pursue a route that would enable divergent parallel synthesis at the penultimate step of the synthetic sequence (Scheme 1). Following identification of a lead compound this paper also will describe approaches to a shortened sequence to reduce the overall cost for our advanced antimalarial compound (**5f**).

Our initial route commenced with acetate protection of the commercially available 4-(4-hydroxyphenyl)cyclohexanone **4a** followed by formation of the gem dihydroperoxide **4b**, under acid-catalysed conditions.[12] After work-up and without any further purification, this intermediate was subjected to a cyclisation reaction with adamantanone in the presence of $Re_2O_7$ according to a procedure previously published by O'Neill et al. to give the acetate tetraoxane **4c** in 46% yield.[9] In this key reaction 2 mol % of the rhenium oxide catalyst was used and the reaction was complete within 2 h. It is thought that this catalyst stabilises the reactive bishydroperoxide intermediate in addition to activating the adamantanone.[13] Phenol acetate protected tetraoxane **4c** was hydrolysed with LiOH and alkylation of the resultant **4d** with allyl bromide[14] and ozonolysis provided aldehyde **4e**. Reductive amination of **4e** with a range of cyclic amines delivered target molecules **5a**–**f** in excellent overall yields. The analogous three carbon-linked analogues were available, by a similar route, by alkylation of phenol **4d** with 4-bromo butene and elaboration as shown in Scheme 1B.

Table 1 records the antimalarial activity of selected analogues. All tetraoxanes were active in the nanomolar region and they all outperformed artesunate following a single oral dose of 30 mg/kg. E209 (**5f**) displayed the best activity in this model with a 66% cure rate and an average mean survival time of 26 days (control animals MST=4 days in this study). Based on the in vitro and in vivo activity (and additional PK analysis, data not shown) analogue **5f** was selected as our lead compound. Three approaches were then investigated to optimise and scale up the synthesis of this compound.

The original synthesis (Scheme 1) was a seven-step process involving the use of an ozonolysis step. To produce a more cost effective synthetic route to E209, we proposed a much simpler route (Scheme 2A) that utilises inexpensive reagents, allowing for the production of **5f** (E209) in a shorter, four-step sequence. This approach is based on the elegant synthetic process reported by Ley for the synthesis of OZ439 which utilises flow chemistry.[16] In their approach, a direct alkylation of chloroacetyl morpholine and subsequent conversion of the amide to the amine led to OZ439 in an 86% yield when $Zn(OAc)_2$ and triethoxysilane were used for the key amide reduction step (Scheme 2A).[17,18] These conditions provide a promising precedent for the use of similar conditions in the production of E209.

Starting with acetate **4c** we were able to prepare the alkylated product **4f** in a one-pot reaction as shown in Scheme 2B. This sequence involves acetate hydrolysis and alkylation in the presence of tetrabutylammonium hydrogen sulfate as a phase transfer catalyst.

For the reduction step we explored a range of silanes with 10 mol % of $Zn(OAc)_2$ and 3 equiv of hydrosilane, but in each case only starting material was recovered. Increasing the concentration of the catalyst to 30 mol % and leaving for 3 days had no effect. The more powerful reducing reagent $LiAlH_4$ was also tested however, this reagent led to a variety of side products due to cleavage of the peroxide bond.

Alternative reagents compatible with selective reduction of tertiary amides such as $Tf_2O/NaBH_4$, $Zn(OAc)_2/HSi(OEt)_3$, $BF_3{\cdot}Et_2O/NaBH_4$, and $Zn(Et)_2$/PMHS/LiCl were based on our previous experience with semi-synthetic artemisinin and tetraoxane chemistry.

We initially examined $Tf_2O$/NaBH4 for the reduction as described by Xiang et al.[19] The proposed mechanism (Scheme 3) involves activation of the amide with trifluoromethanesulfonic anhydride ($Tf_2O$) to form a highly electrophilic iminium triflate which is susceptible by sodium borohydride to produce an *N,O*-acetal. The acetal then eliminates –OTf, assisted by the nitrogen lone pair to form a new iminium ion which is trapped by a second hydride, yielding the target amine. Studies have shown these conditions are highly chemoselective for tertiary amides over other functional groups, which is what is required to avoid cleavage of the tetraoxane unit.[20] Initially we examined the reaction of amide **4f** with 1.1 equiv of $Tf_2O$ and 1.3 equiv of $NaBH_4$ which delivered E209 in a yield of 28%. The amide carbonyl was effectively reduced with