Available online at www.sciencedirect.com
SCIENCE DIRECT®
Bioorganic & Medicinal Chemistry Letters
Bioorganic & Medicinal Chemistry Letters 16 (2006) 2991–2995

Diels–Alder/thiol–olefin co-oxygenation approach to antimalarials incorporating the 2,3-dioxabicyclo[3.3.1]nonane pharmacophore

Paul M. O'Neill,[a,*] Edite Verissimo,[a] Stephen A. Ward,[b] Jill Davies,[b] Edward E. Korshin,[c] Nuna Araujo,[d] Matthew D. Pugh,[a] M. Lurdes S. Cristiano,[d] Paul A. Stocks[a] and Mario D. Bachi[c,*]

[a]*Department of Chemistry, University of Liverpool, PO Box 147, Liverpool L69 3BX, UK*
[b]*Department of Organic Chemistry, Liverpool School of Tropical Medicine, Pembroke Place, Liverpool L3 5QA, UK*
[c]*The Weizmann Institute of Science, Rehovot 76100, Israel*
[d]*University of the Algarve, FCT, Faro P-8000, Portugal*

Received 11 January 2006; revised 22 February 2006; accepted 22 February 2006
Available online 9 March 2006

Abstract—A Diels–Alder/thiol–olefin co-oxygenation approach to the synthesis of novel bicyclic endoperoxides **17a**–**22b** is reported. Some of these endoperoxides (e.g., **17b**, **19b**, **22a** and **22b**) have potent nanomolar in vitro antimalarial activity equivalent to that of the synthetic antimalarial agent arteflene. Iron(II)-mediated degradation of sulfone-endoperoxide **19b** and spin-trapping with TEMPO provide a spin-trapped adduct **25** indicative of the formation of a secondary carbon centered radical species **24**. Reactive C-radical intermediates of this type may be involved in the expression of the antimalarial effect of these bicyclic endoperoxides.

The 2,3-dioxabicyclo[3.3.1]nonane system **1** was first identified in the naturally occurring antimalarial yingzhaosu A **2**.[1,2] It was subsequently incorporated into synthetic antimalarials as Hoffmann LaRoche's arteflene **3**,[3] endoperoxides **4** synthesized by O'Neill et al.[4] β-sulfanyl and β-sulfonyl-endoperoxides **5**–**9b** synthesized by Bachi and co-workers,[5–8] and related cyclic peroxides synthesized by Nojima and co-workers,[9] to become a well-established pharmacophore. C(4)-Methyl-substituted β-sulfanyl- and β-sulfonyl-2,3-dioxabicyclo[3.3.1]nonane derivatives such as **5**–**8** are now readily available by a useful method based on the adaptation of the thiol–olefin co-oxygenation (TOCO) reaction[10] to limonene.[5–8] This method proved to be particularly efficient in a key step of the synthesis of C(4)-phenyl-substituted benzylidene endoperoxides like **10** and **11**[11] (Fig. 1).

More than 50 compounds of types **5**–**8** have been screened for in vitro activity.[12] Ten of the β-sulfonyl-endoperoxides of types **6** and **8** have IC_{50} values lower than 25 nM against *Plasmodium falciparum* (NF54).[12] Upon subcutaneous administration, four β-sulfonyl endoperoxides were shown to be highly active antimala-

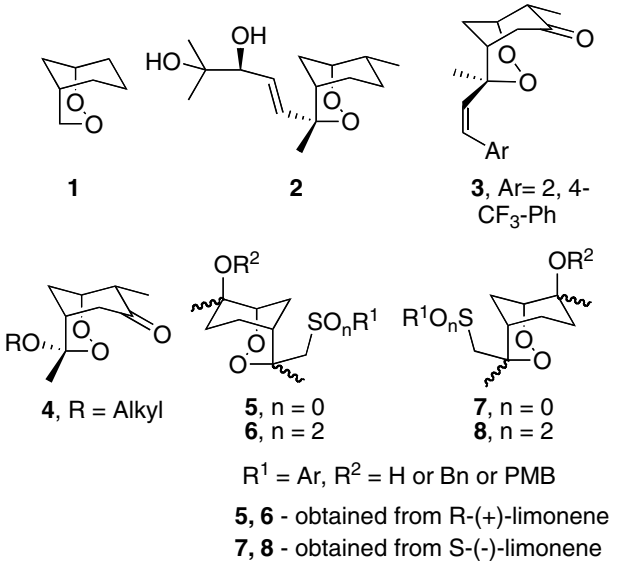
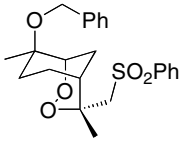


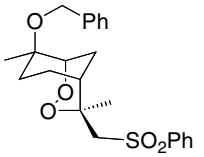
Figure 1. Bicyclic endoperoxides based on the 2,3-dioxabicyclo[3.3.1]nonane pharmacophore.

Keywords: Artemisinin; Arteflene; Endoperoxide; Malaria; Mechanism of action.
* Corresponding authors. E-mail: P.M.oneill01@liv.ac.uk

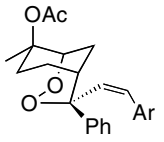
0960-894X/$ - see front matter © 2006 Elsevier Ltd. All rights reserved.
doi:10.1016/j.bmcl.2006.02.059



9a. IC50 = 6.5 nM (NF54)
Artemisinin IC50 = 8.9 nM
ED50 = 0.8 mg/kg
Artemisinin ED50 = 0.95 mg/kg



9b. IC50 = 9.4 nM (NF54)
Artemisinin IC50 = 8.9 nM
ED50 = 0.43 mg/kg
Artemisinin ED50 = 0.95 mg/kg



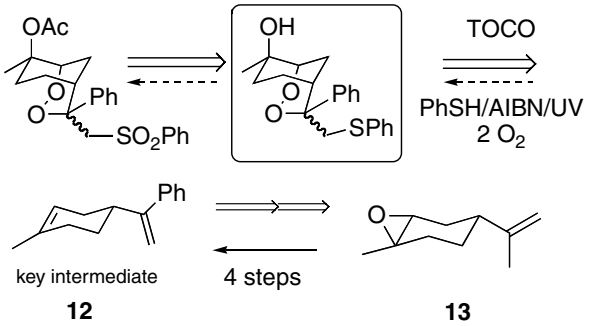
10. Ar = Ph
IC50 = 34 nM (K1)
11. Ar = *p*-Cl-Ph
IC50 = 23 nM (K1)

Figure 2. Structures and antimalarial activities of lead endoperoxides **9a**, **9b**, **10** and **11**.

rials in vivo against *Plasmodium yoelii* and *Plasmodium berghei* strains of malaria parasites. Relative to artemisinin, the most potent compounds **9a** and **9b** (Fig. 2) were about two times more efficacious against chloroquine-sensitive *P. berghei* and 3–5 times more efficacious against chloroquine-resistant *P. yoelii*. Thus, the potency of the endoperoxides **9a** and **9b** is comparable to those of some of the best currently used antimalarial drugs, including artemether and arteether. The benzyloxy derivative **9b** (Fig. 2) exhibits also a reasonable oral antimalarial efficacy about twice the level of arteflene. Antimalarial benzylidene endoperoxides, such as compounds **10** and **11**, have been shown to liberate chalcones following iron-dependent bioactivation of the endoperoxide bridge within isolated digestive vacuoles of *P. falciparum*. Such compounds are considered as pro-drug prototypes.[11]

In this paper, we report on the synthesis of a new series of bicyclic C(4)-phenyl-substituted β-sulfanyl/sulfonyl endoperoxides and the assessment of their antimalarial activity.

The key intermediate in our earlier[11] synthesis of pro-drug prototypes **10** and **11** was the phenyl limonene derivative **12** prepared in four steps from the unsaturated epoxide **13** (Scheme 1). We decided to investigate a more direct and flexible synthesis of **12** by employing a $Sc(OTf)_3$-catalysed Diels–Alder reaction of 1-phenyl-prop-2-en-1-one (**15**) with isoprene. Studies by Kobayashi have shown that scandium perfluoroalkane sulfonates catalyse the reaction of vinyl ketones with isoprene leading to excellent yields of the corresponding cycloadducts with very high levels of regioselectivity.[13]



Scheme 1. Retrosynthetic analysis on sulfone endoperoxides to limonene epoxide **13**.

Enone **15** was prepared from 3-chloropropiophenone **14** by base-catalysed elimination of HCl in 90% yield. The enone product was used immediately in the Diels–Alder reaction with isoprene; the Kobayashi protocol gave the desired product **16** in 76% yield following purification by flash column chromatography; only minor quantities (<2%) of the regioisomeric 1,3-adduct could be detected. Compound **16** was subjected to a Wittig reaction, with methyl triphenyl phosphonium bromide and potassium *tert*-butoxide as base, to give the desired product **12** in 90% yield. Exposure of **12** to optimised conditions for the TOCO reaction gave a mixture of two diastereomers **17a**[14] and **17b**[15] in yields of 70% on a 2g scale.[11] The individual racemic diastereomers were separated by column chromatography and oxidised with *m*-CPBA to give the corresponding sulfones **19a**[16] and **19b**[17] in excellent yields. The TOCO reaction of **12,** performed using *p*-chlorothiophenol instead of phenylthiol, afforded the *p*-chloro-substituted analogues **18a** and **18b**, although in lower yields.

Since previous SAR studies involving C(4) methyl-substituted endoperoxides like **5**–**8** of both the '**a**' and '**b**' diastereomeric series revealed that acetylation of the tertiary alcohol led to improvement in antimalarial activity both in vitro and in vivo;[12] hydroxy endoperoxides **17a** and **17b** were transformed in good overall yield into the corresponding acetoxy sulfides **21a** and **21b** as shown in Scheme 3. The corresponding sulfones **22a** and **22b** were obtained as before, by the use of *m*-CPBA as oxidant. The assignment of stereochemistry at the C-4 position for **22b** has previously been confirmed by a combination of NMR spectroscopy and X-ray crystallography.[7,11]

Prior to testing, we considered the issue of enantiomeric purity since all of the compounds prepared in this study are racemic. Previously, several studies have confirmed that enantiomeric pairs of endoperoxides have identical antimalarial activity; these compounds include antimalarially potent 1,2,4-trioxanes,[18a,b] endoperoxides[18c] and analogues of the present series of bicyclic endoperoxides.[12] Thus, for the purposes of identifying lead endoperoxides, we note that many papers in the literature have employed primary screening of racemic endoperoxides and feel that this is a validated approach to antimalarial lead compound discovery.[18a–c] The antimalarial activity of selected endoperoxides was measured in red blood cell-based assays. Efficacy was monitored by parasite {^{3}H}-hypoxanthine incorporation