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A single, low, oral dose of a 5-carbon-linked trioxane dimer orthoester plus mefloquine cures malaria-infected mice

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ABSTRACT

Four 5-carbon-linked trioxane dimer orthoesters (**6a–6d**) have been prepared in 4 or 5 chemical steps from the natural trioxane artemisinin (**1**). When administered orally to malaria-infected mice using a single dose of only 6 mg/kg body weight along with 18 mg/kg of mefloquine hydrochloride, trioxane dimer orthoester sulfone **6d** completely and safely cured the mice; after 30 days, the cured mice showed no detectable parasitemia, gained at least as much weight as the control mice (no infection), and behaved normally.

Many standard antimalarial drugs like chloroquine are no longer effective due to widespread resistance.[1] A new non-alkaloid class of antimalarial artemisinin trioxanes,[2–9] based on ancient Chinese folk medicine, is now recommended by the World Health Organization (WHO) and is being adopted widely[10–16]; artemisinin combination therapy (ACT) features the very rapid clearance of most of the parasites by the trioxane drug followed by the prolonged antimalarial action of the partner drug. Typically, fixed dose combinations are used,[17] with the most popular example being a curative six dose regimen of a 1:6 fixed combination of artemether (total 320 mg) and lumefantrine (total 1920 mg). Often, however, patient compliance with a repeated-dose regimen is problematic. Therefore, a single dose cure of malaria-infected humans is highly desirable. Toward this goal, we have developed some trioxane monomers[18] and dimers[19] able to cure malaria-infected mice using only a single low oral dose combined with mefloquine. We recently reported a new series of 5-carbon-linked trioxane dimers **4** which, combined with mefloquine, cure malaria-infected mice.[20] We report here a new series of 5-carbon-linked trioxane dimer orthoesters **6a–6d**.

C-10 Acetoxy artemisinin **2b**, prepared in nearly quantitative yield by reducing and then acetylating artemisinin ((**1**→**2a**→**2b**),[21] reacted with silylated 5-carbon-linker **3** to form 10β,10β-dimer allylic alcohol **4** as the major product in 65% yield (Fig. 1).[20] Scale up is not expected to be problematic. Dithexylborane hydroboration of bis-allylic alcohol **4** followed by basic oxidation produced 1,3,5-triol **5** as the major product in 50% yield. Acyclic 1,3,5-triol **5** reacted with three equivalents of a series of commercial orthoesters to form trioxane dimer orthoesters **6a–6c** in 75–91% yields. The stereochemistry of triol **5** was clarified by X-ray crystallography of phenyl orthoester **6b**, establishing its structure as shown in Figure 2. X-ray coordinates have been deposited with the Cambridge Crystallographic Data Center (CCDC# 789228). Trioxane dimer orthoester sulfone **6d** was prepared by thiophenoxide displacement of the bromine atom in bromo orthoester **6c** followed by sulfide→sulfone oxidation. It is noteworthy that thiophenoxide accomplishes this substitution reaction without cleaving the antimalarially crucial peroxide bond in trioxane **6c**; peroxides generally are easily cleaved by thiolate anions.[22] Displacement of bromo orthoester **6c** with sodium benzene-sulfinate gave orthoester sulfone **6d** directly in 84% yield and in >99% purity by HPLC analysis. Trioxane dimer orthoester sulfone **6d** in the absence of solvent is stable at 60 °C for at least one week, with less than 5% decomposition detected by ^{1}H NMR spectroscopy. Orthoesters like **6**, derived from 1,3,5-triols, are considerably more stable toward acid hydrolysis than the corresponding trialkyl orthoesters.[23]

Orthoester sulfone **6d** is stable to simulated stomach acid[24] (pH 2) for at least 24 h at 25 °C as determined by analytical thin layer chromatography.

Each trioxane dimer **6a**–**6d** (0.48 mg) was dissolved in 0.08 mL of 7:3 Tween 80/ethanol and then diluted with 0.73 mL of distilled water for oral administration to 5-week old, approximately 20 g C57BL/6 J male mice (from the Jackson Laboratory) weighing

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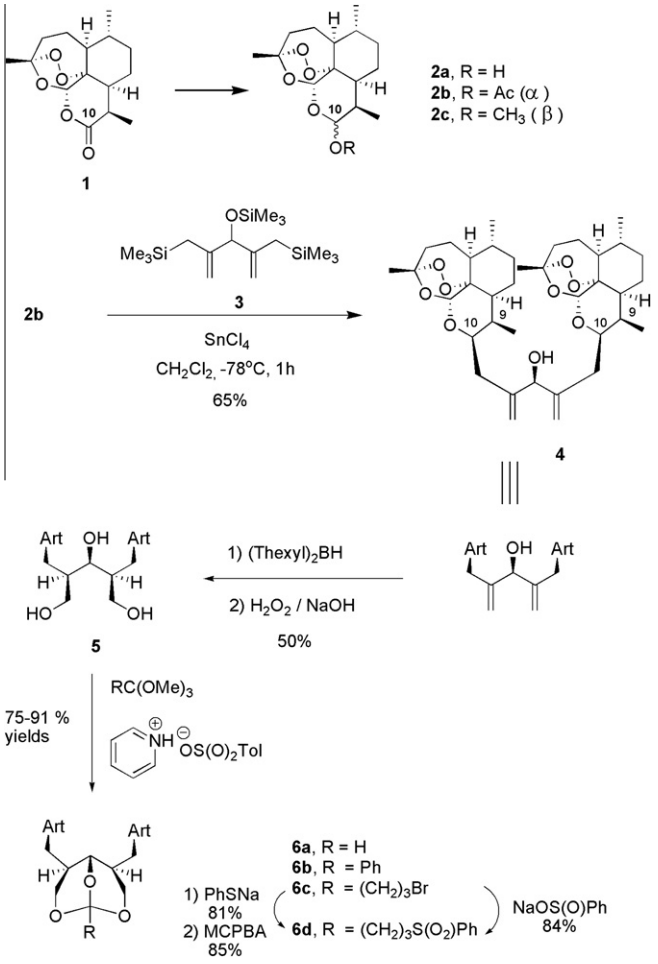

Fig. 1. Trioxane dimer orthoesters.

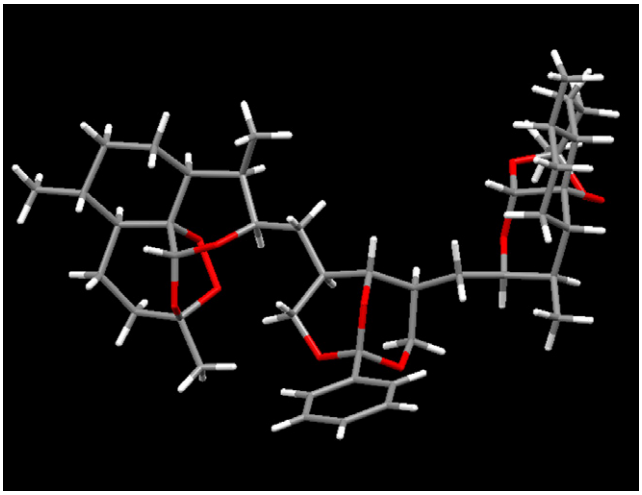

Fig. 2. X-ray crystallography of phenyl orthoester **6b**.

about 20 g that were infected intraperitoneally on day 0 with the *Plasmodium berghei*, ANKA malaria strain (5×10^7 parasitized erythrocytes).

Each of three mice in a group was treated orally 24 h post infection with a single dose of 0.20 mL (0.20 mL/0.81 mL × 0.48 mg = 0.12 mg) of diluted compound solution, corresponding to a dose of 6 mg/kg, combined with 18 mg/kg of mefloquine hydrochloride. Both determining blood parasitemia levels as well as monitoring the duration of animal survival compared to survival time of infected animals receiving no drug were the malariometrics used.

Table 1
Antimalarial efficacy using a single oral dose (6 mg/kg) of trioxane combined with mefloquine hydrochloride (18 mg/kg) in *Plasmodium berghei*-infected mice

Trioxane	Average survival (days) after infection	% Suppression of parasitemia on day 3 post infection
Artemether (**2c**)	14.3 (11, 15, 17)	>99.9
Artemether (6 mg/kg) + lumefantrine (18 mg/kg)	24.0 (23, 23, 26)	>99.99
5	18.6 (12, 16, 30)	>99.9
6a	19.0 (11, 16, 30)	>99.9
6b	23.7 (19, 19, 30)	>99.9
6c	20.7 (12, 22, 30)	>99.99
6d	30 [30, 30, 30]	>99.9
Controls		
Vehicle (no drug)	7.3 (7, 7, 8)	0
Mefloquine (18 mg/kg)	20.0 (16, 17, 28)	>99.9
Lumefantrine (18 mg/kg)	11.7 (11, 12, 12)	99.1

Three days after infection, an average of 9% blood parasitemia (Giemsa microscopy) was observed in the no-drug control group of mice. The average survival time of the malaria-infected animals receiving no drug was 7.3 days post infection.

All of the infected mice in this study receiving trioxane drug artemether (**2c**) plus mefloquine died on the average on day 14 post infection. Importantly, a single oral dose of artemether (6 mg/kg) plus lumefantrine (18 mg/kg) was not curative (Table 1). With mefloquine hydrochloride alone at a single oral dose of 18 mg/kg, the average survival time of the infected mice was 20 days. A widely accepted indication of complete cure (i.e., 100% efficacy) is survival of animals to day 30 post infection with no detectable malaria parasites in the animals' blood at that time.

The average survival times of orthoester-treated infected mice are shown in Table 1. Noteworthy is that orthoesters **6a**–**6c** plus mefloquine were not curative. In sharp contrast, orthoester sulfone **6d** plus mefloquine was fully curative (Table 1). It is apparent from the data presented in Table 1 that the trioxane dimer orthoester sulfone **6d**, at a single oral dose of only 6 mg/kg plus 18 mg/kg of mefloquine hydrochloride, is much higher in efficacy than the antimalarial drug **2c** plus mefloquine and is fully efficacious at curing the malaria-infected mice; all three mice in this 30-day surviving group were completely cured (no parasites in their blood on day 30 post infection), and they had gained at least as much weight as the uninfected control mice (data not shown). All of the orthoesters **6a**–**6d**, as well as trioxane drug **2c**, caused at least 99.9% suppression of parasitemia on day 3 post infection. Neither overt toxicity nor behavioral change attributable to trioxane drug administration was observed in any of the malaria-infected animals cured by trioxane orthoester sulfone **6d** plus mefloquine hydrochloride combination.

In conclusion, syntheses of trioxane dimer orthoesters **6** were achieved in moderate overall yields from the natural trioxane artemisinin (**1**); scale-up synthesis to kilogram quantities of these thermally and hydrolytically stable new chemical entities is expected to be straightforward. The single oral dose antimalarial efficacy of trioxane dimer orthoester sulfone **6d** combined with mefloquine hydrochloride is superior to that of the popular clinically used monomeric trioxane drug **2c**.[25,26] Investigation of the preclinical pharmacology of trioxane dimer orthoester sulfone **6d** will allow a fuller comparison of the chemotherapeutic value of this semi-synthetic endoperoxide versus that of the popular antimalarial trioxane drug **2c**.

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Supplementary data

Supplementary data (experimental details and spectroscopic data for **5** and **6a**–**6d**) associated with this article can be found, in the online version, at doi:10.1016/j.bmcl.2010.09.123.

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