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Antimalarial chemotherapy: Orally curative artemisinin-derived trioxane dimer esters

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Antimalarial chemotherapy; Trioxane dimer esters; Single oral dose ACT; Oral bioavailability

Malaria kills more than 600,000 people, mostly children, each year.^{1–3} No vaccine has been developed yet to fully prevent people from becoming infected with malaria parasites.^{4–6} Antimalarial chemotherapy using nitrogen-containing heteroaromatic compounds like chloroquine and mefloquine has been used successfully for many years to cure malaria-infected people.^{7–10} In recent years, however, widespread resistance of malaria parasites has developed to many of these heteroaromatic drugs.^{11–13} Therefore, new classes of antimalarial drugs are desperately needed. A breakthrough occurred in the early 1970's with the discovery in China that artemisinin (**1**), a naturally occurring endoperoxide sesquiterpene lactone, is strongly efficacious as an antimalarial.^{14–15} Several short-lived, artemisinin-derived 1,2,4-trioxanes have been prepared and some, especially artemether (**2**) and artesunate (**3**), are currently used combined with long-lived, nitrogen-containing antimalarials.^{16–22} Such artemisinin combination therapy (ACT) is recommended as standard chemotherapeutic protocol by the World Health Organization (WHO).²³ Typically, multidose regimens of ACT daily for several days are needed to achieve a complete cure of malaria-infected people.^{16–20} A major problem arises, however, when infected individuals stop taking the ACT prematurely, thereby leading often to parasite recrudescence. Therefore, a major goal of modern antimalarial chemotherapy is to develop new

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Supplementary Data:

Supplementary data (experimental and tabular spectral data) associated with this article can be found in the online version.

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endoperoxides capable of single oral dose ACT cures. Toward this goal, many simple endoperoxides^{24–32} and artemisinin-modified trioxanes^{33–43} have been synthesized and evaluated for antimalarial effectiveness.

Substituted pyridines are important structural units in various pathogen-resistant agrochemicals^{44–50} and in some pharmaceutical drugs.^{51–54} We converted artemisinin (**1**) into 3-carbon linked dimer primary alcohol **4** in 65% overall yield (Scheme 1).⁵⁵ Esterification of primary alcohol **4** without compromising the endoperoxide pharmacophore formed 3-carbon-linked pyridine containing dimer isonicotinate ester **5a** (Scheme 1).⁵⁵ Dimer isonicotinate **5a** had high in vitro antimalarial potency: IC₅₀ = 1.7 nM vs IC₅₀ = 9 nM for artemisinin (**1**).⁵⁵ Based on the structure of this potent antimalarial dimer isonicotinate ester **5a**, we performed and report here a structure-activity (SAR) study featuring artemisinin-derived 3-carbon-linked trioxane dimer esters **5** with diverse substituents on the pyridine ring and on the phenyl ring (Scheme 1).⁵⁶

Antimalarial efficacy data in mice are more valuable and more demanding for preclinical drug development than *in vitro* potency data. Based on our experience with trioxanes we conclude that, within a family of antimalarial trioxanes, *in vitro* potency (IC-50) data do not precisely predict levels of *in vivo* efficacy. Thus, we chose to explore our antimalarial trioxane dimer esters **5** directly by ACT in malaria-infected mice.

Stock solutions were prepared by dissolving mefloquine hydrochloride (1.8 mg) in 93.9 μ L of 7:3 Tween 80:ethanol. This solution was added to 0.6 mg of dimer ester **5** in a 1 dram vial. After approximately 18 h at room temperature, 906.1 μ L of deionized water was added, and then 200 μ L of this stock solution was administered by oral gavage one day post infection to 5-week old C57BL/6J male mice (from Jackson Laboratory) that weighed approximately 20 g, which had been infected with *Plasmodium berghei* ANKA strain (1.5×10^7 parasitized erythrocytes), corresponding to a dose of 6 mg/kg of trioxane dimer in combination with 18 mg/kg of mefloquine hydrochloride.

Antimalarial efficacy results are shown in Table 1. Several important SAR conclusions stand out. As a positive control, artemether (**2**, 6 mg/kg) plus mefloquine hydrochloride (18 mg/kg) prolonged mouse survival to an average of 20.8 days. Dimer primary alcohol **4** and all of the dimer esters **5** were more efficacious than artemether (**2**) plus mefloquine. Difluorobenzoate **5g** prolonged two mice survival until day 30 with only one of the mice having no detectable parasitemia on day 30. Dichlorobenzoate **5h** prolonged mouse survival until at least day 30, with three of the four mice having no detectable parasitemia on day 30 and looking and acting healthy. Survival until day 30 post infection with no parasitemia is widely considered to be a cure. Dichloronicotinate **5i** (two cures) outperformed isomeric dichloroisonicotinate ester **5c** (one cure), even though both **5i** and **5c** have the same log *P* value of 9.5.⁵⁷ Two of four mice treated with dichloronicotinate **5i** had no detectable parasitemia and appeared healthy on day 30. Both esters **5h** and **5i** are hydrolytically stable at pH = 2 and 37 °C, in acetonitrile and water, for at least 96 hours.⁵⁸ The easy synthesis of dimer esters **5** and the partial cures achieved by dichloronicotinate **5i** and especially by dichlorobenzoate **5h** deserve further pharmacological study, which is in progress.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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Abbreviations

SAR	structure-activity relationship
ACT	artemisinin combination therapy
DMAP	4-dimethylaminopyridine
EDC	N-(3-Dimethylaminopropyl)-N'-ethylcarbodiimide hydrochloride

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Figure 1.
Artemisinin and first generation derivatives.

Scheme 1.
Three-carbon-linked trioxane dimer esters **5**.

Table 1

In Vivo Antimalarial Efficacy using a Single Oral Dose of Trioxane Dimer (6 mg/kg) Combined with Mefloquine Hydrochloride (18 mg/kg) in *P. berghei*-Infected Mice.

trioxane	Survival after infection (days) ^a	avg survival (days)	% Parasitemia Suppression ^b
4	29, 18, 30, 27	26	>99.9
5a	29, 18, 30, 20	24.3	>99.9
5b	24, 23, 30, 27	26	>99.9
5c	13, 30, 30, 30	25.8	>99.9
5d	29, 25, 30, 30	28.5	>99.9
5e	30, 13, 30, 30	25.8	>99.9
5f	30, 21, 13, 30	23.5	>99.9
5g	29, 27, 30, 30	29	>99.9
5h	30, 30, 30, 30	30	>99.9
5i	30, 30, 30, 30	30	>99.9
controls:			
infected mice (no drug)	6, 6, 6, 7	6.3	0% ^c
artemether (2) plus mefloquine	20, 30, 13, 20	20.8	>99.9
mefloquine alone	13, 29, 18, 23	20.8	>99.9

^a Bold entries indicate best results.

^b Denotes determination on day 3 after infection.

^c An average of 10.3% parasitemia was determined on day 3 after infection.