Mechanism-Based Design, Synthesis, and *in Vitro* Antimalarial Testing of New 4-Methylated Trioxanes Structurally Related to Artemisinin: The Importance of a Carbon-Centered Radical for Antimalarial Activity†

Gary H. Posner,*,‡ Chang Ho Oh,‡,‖ Dasong Wang,‡ Lucia Gerena,§ Wilbur K. Milhous,§ Steven R. Meshnick,⊥ and Wanida Asawamahasadka⊥,#

Department of Chemistry, The Johns Hopkins University, Baltimore, Maryland 21218, Division of Experimental Therapeutics, Walter Reed Army Institute of Research, Washington, D.C. 20307, and Department of Epidemiology, University of Michigan School of Public Health, Ann Arbor, Michigan 48109

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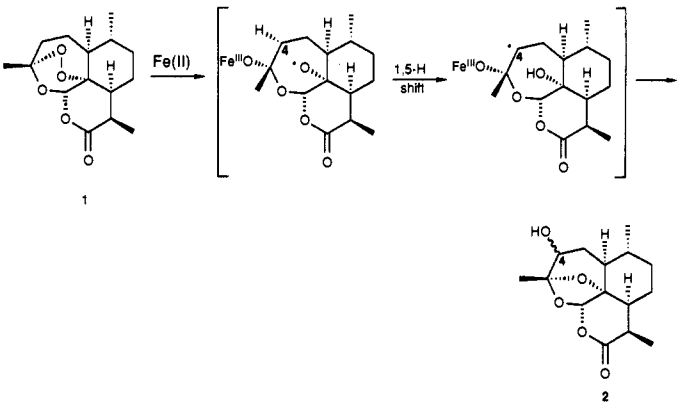
Because malaria parasites are rapidly developing multidrug resistance to the most common chemotherapeutic alkaloidal drugs,[1,2] interest in the antimalarial properties of nonalkaloidal compounds such as the sesquiterpene 1,2,4-trioxane artemisinin (qinghaosu, 1) and its dihydro derivatives is rapidly growing.[3-18] Some of us recently described the design, synthesis, and high *in vitro* and *in vivo* antimalarial potencies of some easily-prepared tricyclic trioxanes structurally related to artemisinin.[19] Also, using one such oxygen-18-labeled trioxane, insight was gained at the molecular level into the mechanism for iron-induced reduction of trioxanes,[20] a process that is considered crucial to the typical physiological pathway involving heme-promoted activation of such trioxanes into metabolites cytotoxic to the malaria parasites.[21] We report here an enlightening test of the proposed mechanism for iron-induced reduction of trioxanes like artemisinin. If, as proposed in Scheme 1, 1,5-hydrogen atom transfer specifically of $H_{4\alpha}$ is a critical step for antimalarial activity and for formation of the typical microbial metabolite hydroxylated dioxolane **2**,[22] then preventing such a 1,5-shift by a structural modification of the trioxane skeleton should effectively shut down this mechanistic pathway and thus also shut down antimalarial activity. Therefore, as a model for dihydroartemisinin, we have prepared monomethylated analogs **3a** and **3b** and *gem*-dimethylated analog **3c** (Scheme 2) and have evaluated their antimalarial potencies *in vitro*.

4-Monomethylated trioxanes **3a** and **3b** and 4,4-dimethylated trioxane **3c**, prepared as outlined in Scheme 2,[19,23] were evaluated *in vitro* against both chloroquine-resistant and chloroquine-susceptible strains of *Plasmodium falciparum* using the semidilution method of Desjardin et al.[24] as modified by Milhous et al.[25] The results are shown in Table 1.

The antimalarial activities shown in Table 1 support the following conclusions: (1) 4β-methylated trioxane **3a** that **can** undergo the 1,5-hydrogen atom transfer shown in Scheme 1 is at least 100 times more potent than 4α-methylated trioxane **3b** that **cannot** undergo such a hydrogen atom transfer; (2) likewise, 4β-methylated trioxane **3a** is at least 100 times more potent also than 4,4-dimethylated trioxane **3c** that **cannot** undergo such a hydrogen atom transfer; and (3) 4β-methylated trioxane **3a** is more potent than artemisinin.

The benzyl ether **3d** was prepared (Scheme 3) as a more lipophilic derivative of 4,4-dimethylated trioxane alcohol **3c** (*cf.* arteether *vs* dihydroartemisinin) and as a close analog of the corresponding 4-unmethylated trioxane benzyl ether that showed excellent antimalarial activity.[19a] Even though 4,4-dimethylated benzyl ether **3d** has ex-

Scheme 1



Scheme 2

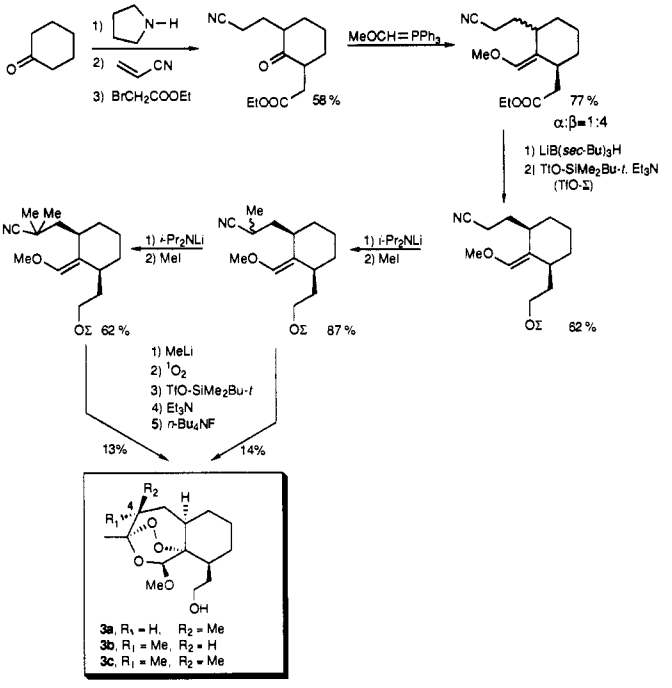


Table 1. *In Vitro* Antimalarial Activity

compound	IC_{50} (ng/mL) W-2 Indochina clone	IC_{50} (ng/mL) D-6 African clone
3a	4.5	3.5
3b	>500	>500
3c	>500	>500
3d	>500	>500
artemisinin (1)	8	8

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‡ The Johns Hopkins University.

§ Walter Reed Army Institute of Research.

⊥ University of Michigan School of Public Health.

‖ Current address: Department of Chemistry, Inje University, Kimhae 621-749, Korea.

Current address: Department of Clinical Microscopy, Faculty of Medical Technology, Mahidol University, Bangkok, 10700, Thailand.

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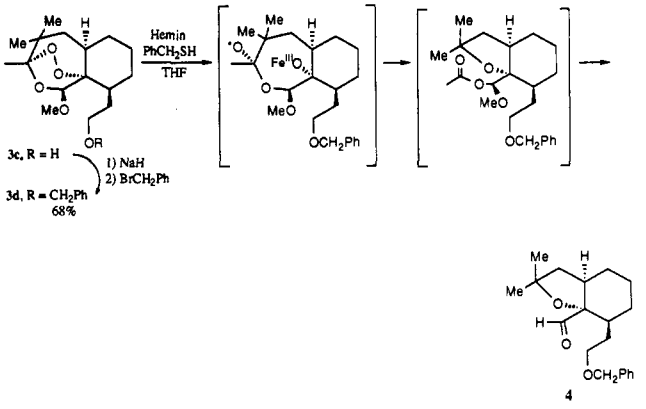
Scheme 3



tremely low antimalarial activity and cannot undergo the 1,5-hydrogen atom transfer depicted in Scheme 1, it is reduced by heme (generated *via* benzyl mercaptan reduction of hemin) in tetrahydrofuran (THF) according to a previously proposed second mechanistic pathway[20] (Scheme 3) to form fragmented aldehyde **4** as the major product.[23] Whether the mechanistic pathway shown in Scheme 3 or in Scheme 1 is followed depends critically on which oxygen atom of the trioxane peroxide linkage becomes associated with the reducing iron atom; this situation is reminiscent of the iron-induced, regiocontrolled, reductive cleavage of the endoperoxide bond in PGH_2 leading to either prostacyclin or thromboxane products.[26]

In conclusion, the virtual lack of antimalarial activity of 4α-methylated trioxane **3b** and of *gem*-dimethylated trioxanes **3c** and **3d** plus the high antimalarial activity of 4β-methylated trioxane **3a** are noteworthy for three reasons: (1) they suggest for the first time that **a reaction pathway proceeding via a carbon-centered radical is likely to be important for the antimalarial activities of some trioxanes like artemisinin**;[27] (2) they highlight the value of mechanistic understanding at the molecular level for the rational design of potent antimalarial trioxanes like **3a**; and (3) they illustrate how one small stereochemical change (*i.e.*, diastereomer **3a** *vs* **3b**) can be used as a molecular on–off switch for antimalarial activity. Such new information may help the rational design of better nonalkaloidal antimalarial agents.

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Supplementary Material Available: Spectroscopic and analytical characterization of trioxanes **3a–d** and of fragmentation product **4** (2 pages). Ordering information is given on any current masthead page.

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