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Antimalarial Endoperoxides that are Potent and Easily Synthesized

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The rapidly increasing resistance of *Plasmodium falciparum* malaria parasites to previously efficacious alkaloidal drugs like chloroquine has prompted a worldwide search for new classes of compounds, not only for malaria chemoprophylaxis but also for chemotherapy, especially of acute malaria (Cumming et al 1996; Meshnick et al 1996). This search has led to the development of the fluorinated alkaloid drugs mefloquine (Sweeney 1981) and halofantrine (Goldsmith 1992) and also to the isolation and characterization of the potent and fast-acting 1,2,4-trioxane artemisinin (qinghaosu, **1**) (Fig. 1), as the active antimalarial component of a plant extract used in China for over two thousand years as a herbal remedy for malaria (Zhou & Xu 1994). Also, this search has recently led to much structure-activity relationship study of 1,2,4-trioxanes (Avery et al 1996) and to an understanding of the fundamental biological (Meshnick et al 1996) and chemical (Cumming et al 1996) mechanisms of actions of such trioxanes.

Malaria parasites self-destruct after encountering a trioxane (Meshnick et al 1996). Triggered by the iron-rich environment inside the malaria parasite, the trioxane is chemically reduced by ferrous ions. This process ruptures the peroxidic oxygen-oxygen bond of the trioxane and forms an oxygen-centered free radical (a reactive oxygen species). Being a reactive intermediate, this oxygen radical can undergo various subsequent chemical transformations. One such transformation that is important for high antimalarial activity is a 1,5-hydrogen atom shift to form a somewhat more stable carbon-centered free radical intermediate (Fig. 2) (Posner et al 1994).

Immediately thereafter, the carbon radical fragments to release a stable organic vinylic ether and a reactive high-valent iron-oxo intermediate (Posner et al 1995). Evidence for this high energy iron-oxo species rests on several different kinds of trapping experiments. This iron-oxo species itself can kill the malaria parasite by oxygenating and thereby disrupting various vital biomolecules inside the parasite, and/or the iron-oxo species can epoxidize the vinylic ether to form an alkylating and therefore damaging epoxide (Fig. 2).

Based on this understanding at the molecular level of the chemical mechanism of artemisinin's antimalarial activity, a structural and synthetically much simpler class of peroxides has been developed. These endoperoxides (not trioxanes) were designed as inexpensive and easily synthesized (two steps from commercial reactants) compounds that were expected to kill malaria parasites (Fig. 3) (Posner et al 1996a). Phenyl endoperoxide **2a**, prepared on a multigram scale, is a crystalline solid that is stable, even at 60°C, for at least 24 h. It does indeed kill malaria parasites; on a nanomolar basis, endoperoxide **2a** has about 12% of the in-vitro antimalarial activity of the complex sequiterpene natural product artemisinin (**1**). Thus, this initial success is proof that the concept is valid;

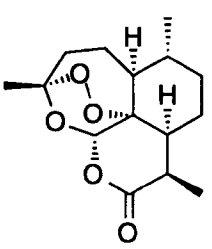


FIG. 1. Chemical structure of artemesinin (qinghaosu).

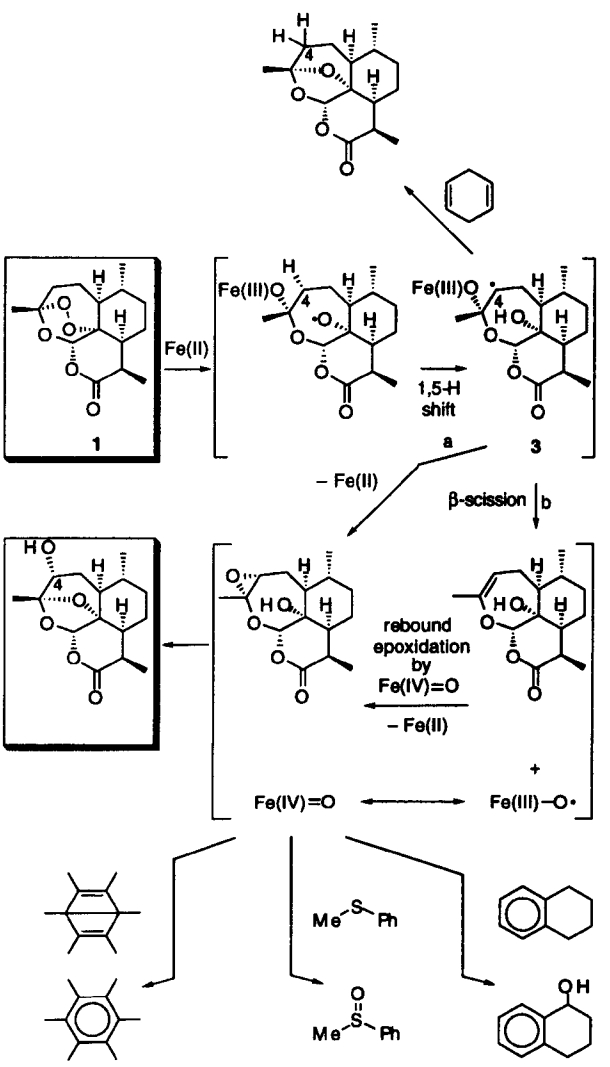


FIG. 2. Mechanism of artemisinin's reduction by iron (II).

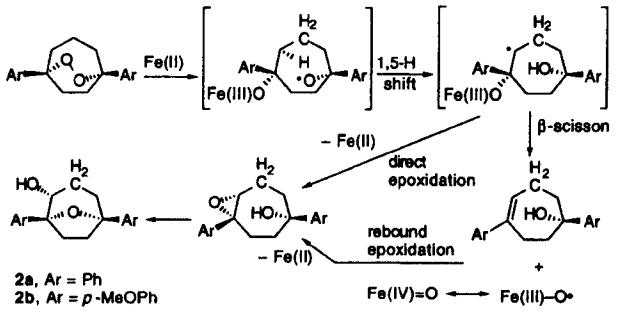


FIG. 3. Synthesis of diaryl bicyclic [3.2.2.] endoperoxides.

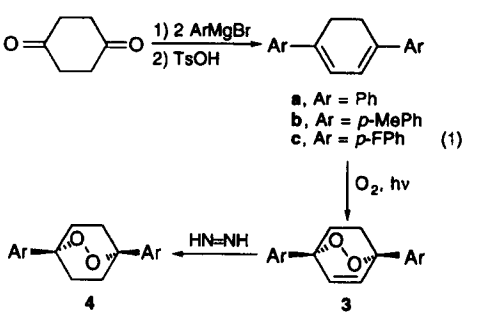


FIG. 4. Synthesis of diaryl bicyclic [2.2.2.] endoperoxides.

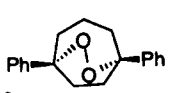
some mechanistically designed, structurally simple, and easily synthesized endoperoxides are indeed antimalarial, and therefore further study to prepare analogs with optimized potency and minimized possible toxicity is clearly worthwhile.

As an initial exploration of its possible toxicity and its possible activity against other parasites, endoperoxide **2a** was examined further. Because humans with compromized immune systems are susceptible to *Toxoplasma gondii* parasites that often cause opportunistic infections such as cerebral encephalitis (Ou-Yang et al 1990), endoperoxide **2a** was examined in-vitro for its activity against *T. gondii* in cultured L929 cells; the results are shown in Table 1 in comparison with the drugs atovaquone and artemisinin (**1**).

At 1.0-μM concentrations, endoperoxide **2a** is approximately comparable to artemisinin in potency. What is especially promising is the therapeutic index (activity/toxicity ratio) characteristic of endoperoxide **2a** at 1.0-μM concentration (Table 1): atovaquone, 95.2 / 17.4 = 5.5; artemisinin, 75.8 / 10.3 = 7.4; endoperoxide **2a**, 69.6 / 0 = ≫ 100. Even at 10-μM concentration, endoperoxide **2a** has no measurable toxicity (Fishwick et al 1995).

Like bicyclic [3·2·2] endoperoxide **2a**, bicyclic [2·2·2] endoperoxides **3** and **4** were prepared very easily and rapidly (Fig. 4) (Posner et al 1996b).

Table 1. Intracellular replication of *T. gondii* in cultured L929 cells in-vitro.

Drug	Activity/toxicity	Dose (μM)	Inhibition at 24 h (% of control)
Atovaquone	activity	0·1	78·4
		1·0	95·2
		16·0	95·7
	toxicity	0·1	15·6
		1·0	17·4
		10·0	18·3
Artemisinin	activity	0·1	29·6
		1·0	75·8
		10·0	75·8
	toxicity	0·1	8·6
		1·0	10·3
		10·0	7·7
 2a	activity	0·1	30·8
		1·0	69·6
		10·0	77·6
	toxicity	0·1	0·3
		1·0	0·0
		10·0	0·0
		50·0	31·2

Data from NIH, Alexandra Fairfield.

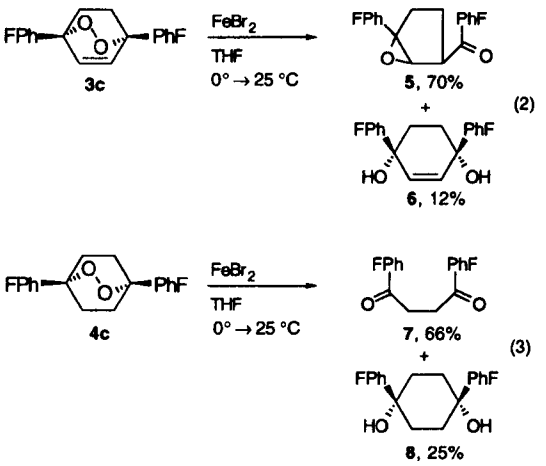


FIG. 5. Reduction of fluorophenyl endoperoxides.

Although the phenyl and the tolyl endoperoxides **3a**, **3b**, and **4a** are not very potent antimalarials, both the unsaturated *p*-fluorophenyl endoperoxide **3c** and the saturated *p*-fluorophenyl endoperoxide **4c** have, on a nanomolar basis, approximately 15% of the antimalarial activity of the structurally complex, natural, clinically used, trioxane artemisinin (**1**). Both inexpensive and easily accessible fluorophenyl endoperoxides **3c** and **4c** are crystalline compounds, stable for at least 40 h at 60°C (Posner et al 1996b). In the presence of ferrous bromide, both fluorinated endoperoxides **3c** and **4c** are reduced rapidly to form products **5–8** (Fig. 5). A plausible mechanism to account for these $FeBr_2$ reductions is depicted in Fig. 6. Reductive cleavage of the weak peroxide bond followed, in pathway a, by a second electron transfer from iron(II) and liberation of two equivalents of Fe(III) produces 1,4-diols **6** and **8**. Carbonyl formation, as in pathway b, releases a carbon-centered radical that fragments to form ethylene and the observed 1,4-diketone **7**; alternatively via pathway b, the unsaturated carbon radical cyclizes and directly forms the observed epoxy cyclopentane ketone **5** as a single diastereomer.

Although reduction products **6–8** showed virtually no in-vitro antimalarial activity when tested as pure compounds, epoxy ketone **5** has measurable antimalarial activity. Thus, unsaturated fluorophenyl endoperoxide **3c** may be a prodrug triggered by iron(II), inside a malaria parasite, to release electrophilic epoxy ketone **5** that itself or, after enolization and epoxide opening, as the isomeric γ-hydroxy-α,β-enone Michael

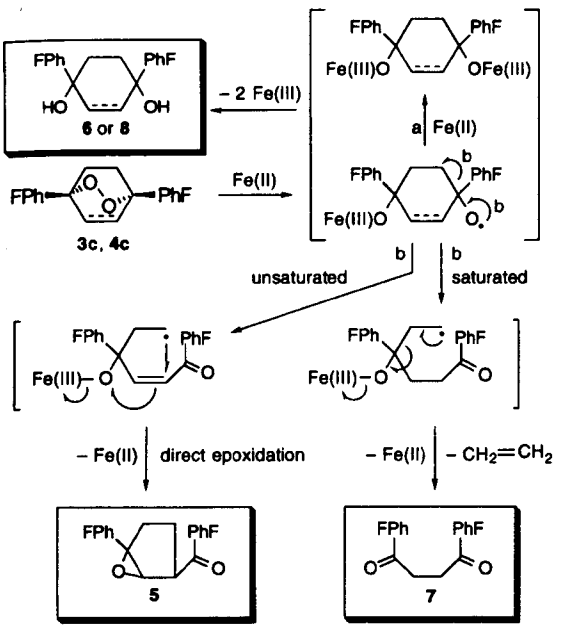


FIG. 6. Mechanism of reduction of fluorinated endoperoxides.

acceptor may kill the parasite. Likewise, saturated fluorophenyl endoperoxide **4c** may be a prodrug, activated by iron(II) to release ethylene that could be oxidized by the malaria parasite's cytochrome oxidase enzymes into ethylene oxide, an extremely reactive and damaging alkylating agent (Ortiz de Montellano 1985).

Saturated bicyclic [2·2·2] endoperoxide **4c** has very recently been found to have measurable activity in an in-vivo rodent malaria model (Peters et al 1993). Thus, this fluorinated endoperoxide **4c**, as well as bicyclic [3·2·2] endoperoxide **2a**, is a prototype suggesting strongly that fine-tuning of chemical structure to maximize the in-vivo antiparasitic therapeutic index is a worthy goal of great practical potential. We are actively pursuing this goal. Small samples of these endoperoxides are available upon request for further biological screening.

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