Pergamon

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Antimalarially Potent, Easily Prepared, Fluorinated Endoperoxides

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Abstract: Three or four step chemical synthesis and *in vitro* antimalarial testing showed crystalline, thermally stable, bicyclic endoperoxides **3c** and **4c** to be potent antimalarials, having approximately 15% of the antimalarial activity of the clinically used, complex, sesquiterpene, lactone, natural product artemisinin (qinghaosu, **1**). A novel mechanism involving reactive alkylating agents is proposed to account for the ability of endoperoxides **3c** and **4c** to kill malaria parasites.
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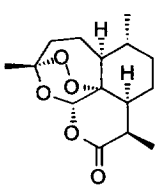
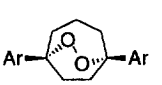
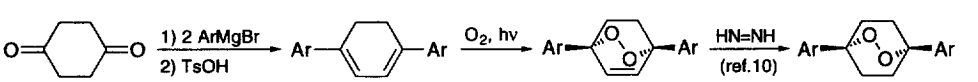
The rapidly increasing resistance of *Plasmodium falciparum* malaria parasites to previously efficacious alkaloidal drugs like chloroquine has prompted a worldwide search for new classes of compounds not only for malaria chemoprophylaxis but also for chemotherapy, especially of acute malaria.[1,2] This search has led to the development of the fluorinated alkaloid drugs mefloquine[3] and halofantrine[4] and also to the isolation and characterization of the potent and fast-acting 1,2,4-trioxane artemisinin (qinghaosu, **1**) as the active antimalarial component of a plant extract used in China for over two thousand years as an herbal remedy for malaria.[5] This search recently has led also to much structure-activity relationship study of 1,2,4-trioxanes[6] and to an understanding of the fundamental biological[1] and chemical[2] mechanisms of actions of such trioxanes. Mechanism-based design, two-step chemical synthesis, and *in vitro* antimalarial testing demonstrated that structurally simple and inexpensive bicyclic [3.2.2] endoperoxides **2** are potent antimalarials, having approximately 15% of the antimalarial activity of the complex sesquiterpene natural product artemisinin (**1**) on a nanomolar basis.[7] Because the similar, symmetrical, unsaturated bicyclic [2.2.2] endoperoxides **3** and saturated analogs **4** are so easily accessible in good yields from commercially available chemicals (eq. 1) and because natural bicyclic [2.2.2] endoperoxide ascaridole is biologically active,[8,9] we prepared a series of such bicyclic [2.2.2] endoperoxides and determined their *in vitro* antimalarial activities (Table I).



artemisinin
(qinghaosu, **1**)



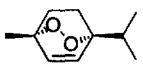
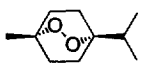
2, Ar = Ph, *p*-MeOPh



a, Ar = Ph
b, Ar = *p*-MePh
c, Ar = *p*-FPh

3 **4** (1)

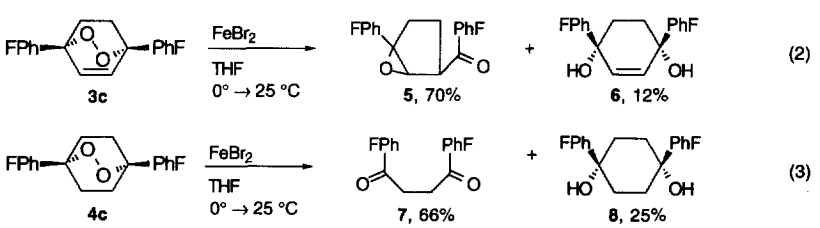
Table I. Chemical Structure-Antimalarial Activity Relationships in Chloroquine-Sensitive *P. falciparum* (NF54)[11] Parasites *in vitro*[a]

Compound	Antimalarial Activity, IC_{50} (nM)
3a	180
3b	140
3c	**70**
4a	210
4c	**63**
 (ascaridole)	650[b]
	190[b]
artemisinin (**1**)	10
chloroquine	5.0

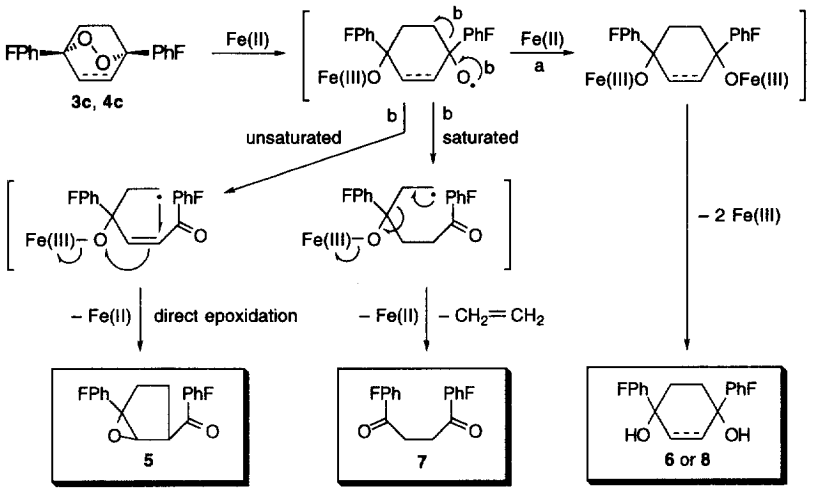
[a]Antimalarial activity was determined by measuring the incorporation of [^{3}H]hypoxanthine, by the method of Desjardins[12] as modified by Milhous.[13] All drug concentrations were assayed in quadruplicate; for these compounds, the coefficients of variation averaged 7.7%. Dose-response curves were fit to the data using the Marquardt algorithm;[14] R^2 values for these curves were ≥0.988.

[b]Assay may underestimate the potency of these volatile compounds.

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**[15] 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 hours at 60 °C. In the presence of ferrous bromide, both fluorinated endoperoxides **3c** and **4c** are reduced rapidly to form products **5**–**8** (eqs. 2 and 3).[15] A plausible mechanism to account for these $FeBr_2$ reductions is depicted in Scheme I. 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**.[15] Carbonyl formation, as in pathway b, releases a carbon-centered radical that fragments to form ethylene (trapped as 1,2-dibromoethane)[16] 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.[15] The absence of rearrangement of hexamethyl Dewar benzene during $FeBr_2$ induced formation of products **5** and **6** from unsaturated endoperoxide **3c** argues against the intermediacy of a high-valent iron-oxo species and thus against a rebound epoxidation mechanism for formation of epoxy ketone **5**.[17]



Scheme I



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,[18] as the isomeric γ-hydroxy-α,β-enone Michael acceptor[19] 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[20] into ethylene oxide, an extremely reactive and damaging alkylating agent.[21,22]

Besides the natural unsaturated bicyclic [2.2.2] endoperoxide ascaridole, several biologically active steroidal unsaturated bicyclic [2.2.2] endoperoxides have been isolated and characterized.[23, 24] Introducing a judiciously positioned fluorine atom is well known in the pharmaceutical industry often to improve the desirable pharmacological properties of a potential drug; in the chemical war against malaria, development of the fluorinated alkaloid mefloquine[3] has been a major success story for chemoprophylaxis of this infectious human disease especially for western travelers to high risk tropical areas. When a person does contract malaria, especially severe cerebral malaria, then the fast-acting trioxane drugs related to artemisinin are becoming more and more clinically useful.[1,2] One such promising lead compound is the fluorinated trioxane Fenozan-50 F.[25] The results recorded here show that easily prepared, inexpensive, fluorinated, antimalarially potent bicyclic [2.2.2] endoperoxides **3c** and **4c** are worthy of further study as new lead compounds in the worldwide fight against malaria. Finally, we suggest here for the first time a novel chemical mechanism, differing greatly from that operating in the bicyclic [3.2.2] endoperoxide series,[7] *via* which antimalarially potent bicyclic [2.2.2] endoperoxides **3c** and **4c** may kill malaria parasites by generating powerful alkylating agents like epoxy ketone **5**[26-28] and ethylene oxide, thereby damaging vital biomolecules inside the parasites.

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