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Mechanism-Based Design of Simple, Symmetrical, Easily Prepared, Potent Antimalarial Endoperoxides

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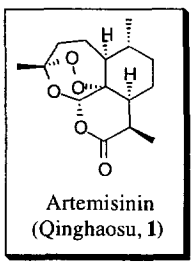
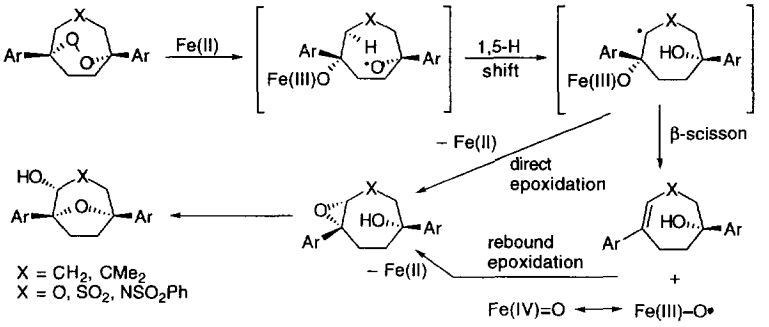
Abstract: Mechanism-based design, two-step synthesis, and *in vitro* antimalarial testing showed thermally stable, crystalline, bicyclic endoperoxides **2a** and **2b** to be potent antimalarials. Their reduction by $FeBr_2$ proceeds *via* oxy-radicals and then carbon radicals that undergo β-scission to form an alkene and a high-valent Fe=O species.

The discovery of a new class of non-alkaloidal and fast-acting antimalarial 1,2,4-trioxanes, exemplified by the natural sesquiterpene endoperoxide artemisinin (qinghaosu, **1**) and its derivatives, has stimulated much synthetic and mechanistic chemical research.[1,2] We have shown recently that *in vitro* reduction of artemisinin (**1**) by ferrous bromide, like the *in vivo* reduction of artemisinin by iron-porphyrins, proceeds *via* an oxygen-centered and then a carbon-centered radical to form a very reactive high-valent iron-oxo species and a potent alkylating epoxide.[3,4] Based on this detailed mechanistic understanding and stimulated by recent findings that two natural non-trioxane endoperoxides indeed have antimalarial activity,[5] we have now designed some structurally simple, symmetrical, easily accessible endoperoxides as inexpensive potential antimalarial drug candidates. This mechanism-based design strategy required endoperoxides that would be reduced by Fe(II) to form first an oxy-radical and then, *via* a 1,5-hydrogen atom shift, a carbon-centered radical and then, *via* radical β-scission, the crucial Fe(III)–O• [↔ Fe(IV)=O] species; then a highly electrophilic (*i.e.* alkylating) epoxide would be formed, with regeneration of Fe(II) (Scheme 1). The symmetry of the designer parent endoperoxides ensured that electron transfer from Fe(II) to initiate this cascade of radical intermediates would generate the same initial oxy-radical intermediate irrespective of which oxygen atom of the endoperoxide accepted an electron from iron. Herein we report that two such simple, symmetrical endoperoxides do indeed show substantial *in vitro* antimalarial activity.

Scheme 1





Bicyclo[3.2.2]nonane endoperoxides **2a**, **2b**, and **3**, prepared according to literature precedent[6] *via* photosensitized oxygenative cyclization of the corresponding 1,6-dienes (eqs. 1 and 2), are stable crystalline compounds; phenyl endoperoxide **2a**, prepared on gram scale, is stable even at 60 °C for at least 24 hours. Endoperoxide sulfone **4**, prepared from α-bromoacetophenone (eq. 3), also is crystalline. Tebbe methylenation[7] of α-bromoacetophenone and reaction of the resultant allylic bromide with either bis(tributyltin)oxide (eq. 4)[6c] or with benzenesulfonamide (eq. 5) produced heteroatom-containing 1,6-dienes that underwent smooth photooxidative cyclization to form ether endoperoxide **5** and sulfonamide endoperoxide **6**.[8] The *in vitro* antimalarial activities of these endoperoxides are presented in Table I.

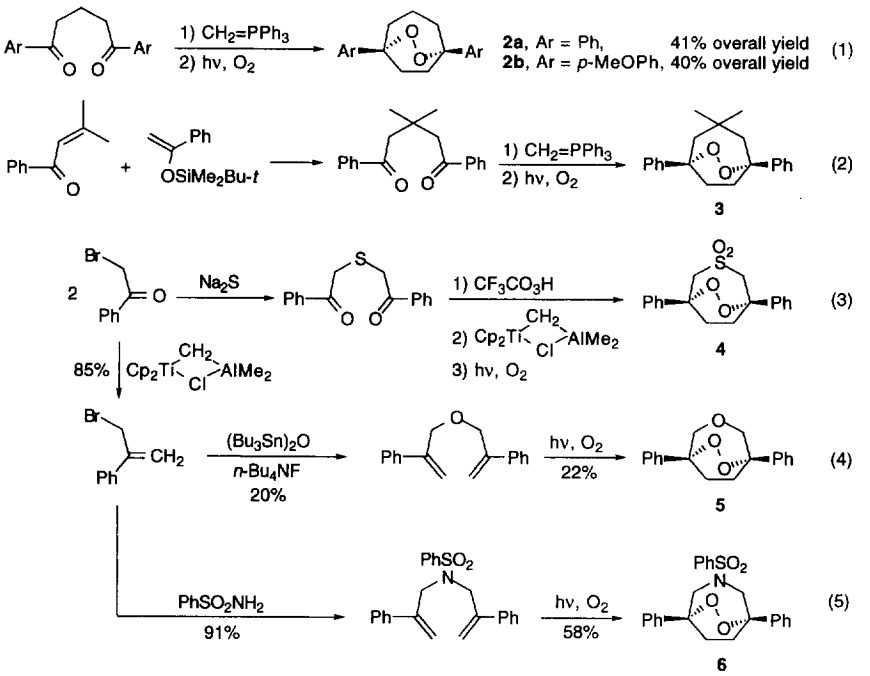


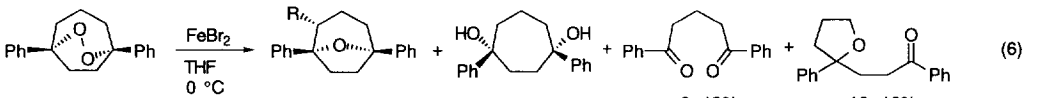
Table I. Chemical Structure-Antimalarial Activity Relationships in Chloroquine-Sensitive *P. falciparum* (NF54)[9] Parasites *in vitro*[a]

Compound	Antimalarial Activity, IC_{50} (nM)
2a	89
2b	62
3	1800
4	>2500
5	>2500
6	>2500
Artemisinin (**1**)	11
Chloroquine	5.0

[a]Antimalarial activity was determined by measuring the incorporation of [^{3}H]hypoxanthine, by the method of Desjardins[10] as modified by Milhous.[11] All drug concentrations were assayed in quadruplicate; the standard deviation for each set of quadruplicates was ≤37% of the mean. Dose-response curves were fit to the data using the Marquardt algorithm;[12] R^2 values for these curves were ≥0.990.

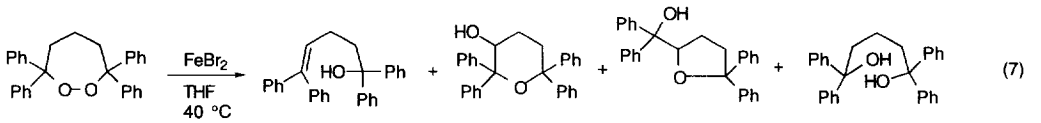
The antimalarial activities (Table I) of these endoperoxides allow the following important generalizations: (1) despite their structural and synthetic simplicity, bicyclic endoperoxides **2a** and **2b** both have high antimalarial activity [about 1/7 that of the complex sesquiterpene trioxane artemisinin (**1**) on a nanomolar basis]; (2) *gem*-dimethyl bicyclic endoperoxide **3** is almost inactive; and (3) none of the endoperoxides **4–6** containing sulfur, oxygen or nitrogen atoms has any significant antimalarial activity.

In accord with the mechanistic expectations outlined in Scheme I, bicyclic endoperoxide **2a** reacted rapidly (within 20 minutes) with ferrous bromide in THF to form the hydroxylated bicyclic ether **7a** (eq. 6), presumably *via* an intermediate epoxide, as depicted in Scheme 1.[3] Furthermore, when this reaction was performed in the presence of excess hexamethyl Dewar benzene, the rearrangement product hexamethylbenzene was produced in 35% yield; this skeletal rearrangement is characteristic of a high-valent iron-oxo intermediate.[3,13]



2a → **7a**, R = HO, 9%; **7b**, R = H, 17% + **8**, 42% + **9**, 13% + **10**, 16% (6)

The first **direct evidence** for olefin formation as shown in Scheme 1 *via* β-scission of Fe(III)–O• from a carbon-centered radical intermediate comes from isolation of the expected olefinic product **12** from Fe(II) reduction of peroxide **11** (eq. 7);[8] a control experiment showed that diol **15** does **not** undergo dehydration to form hydroxy olefin **12** under these reaction conditions. Also, the epoxide of hydroxy olefin **12** is a likely intermediate that would react *via* intramolecular nucleophilic opening to form both tetrahydropyran **13** and tetrahydrofuran **14**. Sterically hindered and sluggishly reactive peroxide **11** lacks any significant *in vitro* antimalarial activity at 37 °C.



11 → **12**, 3% + **13**, 24% + **14**, 8% + **15**, 21% (7)

In conclusion, the high antimalarial potency of endoperoxides **2** was predicted based on recent advances in understanding the fundamental molecular mechanism of antimalarial action of the Chinese trioxane drug artemisinin (**1**).[3] This successful prediction further validates the mechanistic scheme recently proposed for artemisinin's mode of action and encourages preparation of other simple endoperoxides that may possess high antimalarial potency. Further biological evaluation of endoperoxides **2** and mechanism-based design of other simple peroxide antimalarials are underway, and results will be reported in due course.

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