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Communications to the Editor

Further Evidence Supporting the Importance of and the Restrictions on a Carbon-Centered Radical for High Antimalarial Activity of 1,2,4-Trioxanes Like Artemisinin

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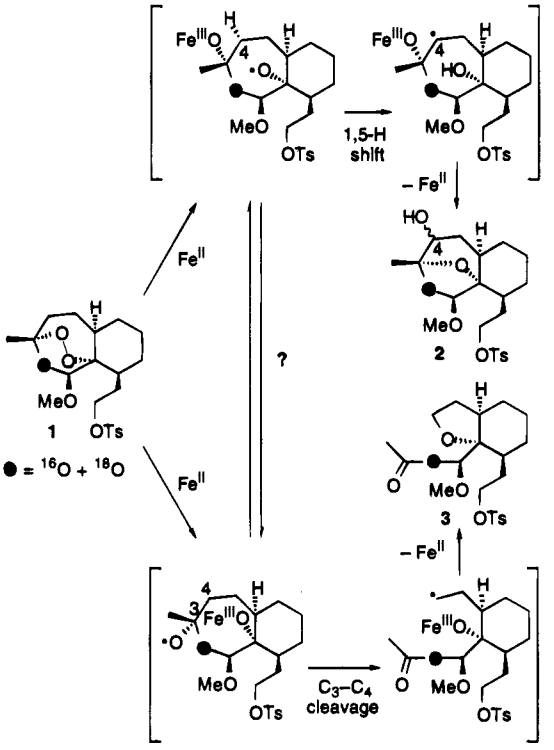
Using regiospecifically oxygen-18-labeled antimalarial 1,2,4-trioxane **1**, we have shown that ferrous ion reduces the crucial peroxide linkage to form oxy radical and then carbon radical intermediates leading to the C_4-hydroxylated product **2** and the ring-contracted product **3** in Scheme 1.[1] Using a stereochemical probe, we have shown further that, of these two pathways, only the first involving a C_4 radical intermediate leading to the C_4-hydroxylated product **2** is important for high antimalarial activity.[2a] Now we report significant and strong further evidence supporting the key role and the limitations of such C_4 radicals in the antimalarial activity of several new tricyclic trioxanes bearing diverse substituents at C_4. The structures and the antimalarial activities of these artemisinin analogs are shown in Table 1, and their syntheses are summarized in Schemes 2 and 3.

The antimalarial data in Table 1 support the following generalizations: (1) like the previously reported C_4-methyl derivative **5**,[2a] the new C_4-benzyl compound **6** and the new C_4-(trimethylsilyl)methyl analog **7** having

Scheme 1



β-stereochemistry[7] at C_4 (thereby allowing the critical H_α atom transfer from the spatially proximate C_4 to the oxy radical in a 1,5-fashion forming a C_4 radical) are at least 12–200 times more active antimalarials than the corresponding α-substituted derivatives **5**–**7**; (2) both $C_{4\beta}$-substituted derivatives **5** and **6** are potent antimalarials, comparable in activity to artemisinin (Table 1) and having 11–13 times higher activity than the C_4-unsubstituted parent **4**, thereby indicating that a tertiary (*i.e.*, more stable) C_4 radical center seems to be better than a secondary C_4 radical center (upper pathway in Scheme 1) at promoting antimalarial potency; (3) likewise, the C_{8a}-unsubstituted $C_{4\beta}$-benzyl analog **9** has significantly higher antimalarial activity than the corresponding C_4-unsubstituted parent analog **8**;[8] and (4) unexpectedly, the incorporation of a $C_{4\beta}$-substituent that substantially stabilizes an adjacent carbon radical

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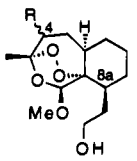
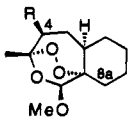
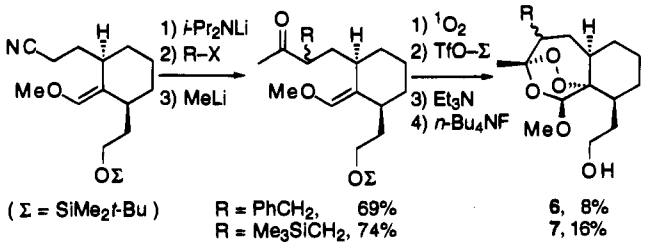
Table 1. Structure–Antimalarial Activity Relationships in Chloroquine-Sensitive *P. falciparum* (NF54)[3] Parasites *in Vitro*[a]

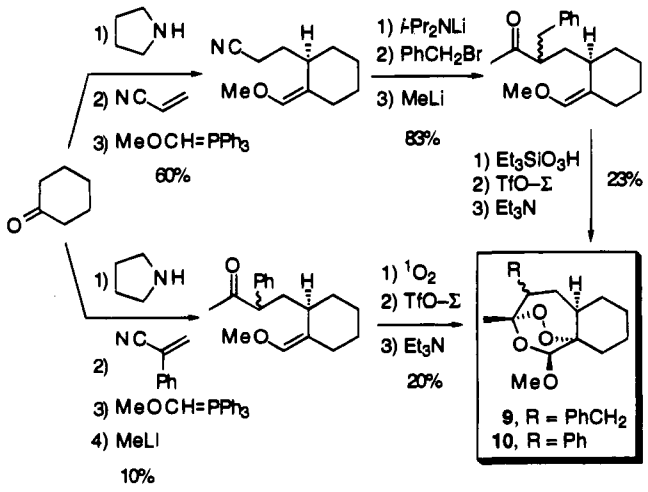
compd	R_β	IC_{50} (ng/mL)	R_α	IC_{50} (ng/mL)
				
4	H	34	H	34
5	Me	2.2	Me	360
6	$PhCH_2$	3.0	$PhCH_2$	600
7	Me_3SiCH_2	86	Me_3SiCH_2	>1000
				
8	H	220		
9	$PhCH_2$	98		
10	Ph	610		
artemisinin		3.0		
chloroquine		2.6		

[a] Antimalarial activity was determined by measuring the incorporation of [^{3}H]hypoxanthine, by the method of Desjardins[4] as modified by Milhous.[5] All drug concentrations were assayed in quadruplicate; the standard deviation for each set of quadruplicates was ≤18% of the mean. Dose–response curves were fit to the data using the Marquardt algorithm;[6] R^2 values for these curves were ≥0.992.

Scheme 2

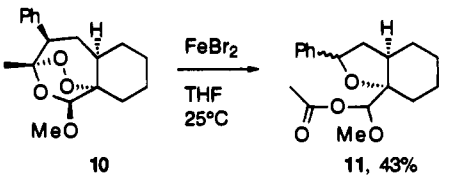


Scheme 3



more effectively than a methyl or benzyl group,[9] as in the $C_{4\beta}$-(trimethylsilyl)methyl analog **7** and the $C_{4\beta}$-phenyl analog **10**, does **not** produce a potent antimalarial analog.[10] All of the $C_{4\beta}$-oriented substituents in these analogs are spatially remote from the α-oriented peroxide linkage and therefore, according to molecular models, cannot interfere with approach of iron to the peroxide linkage.

The surprisingly low antimalarial activity of such $C_{4\beta}$ analogs **7** and **10** prompted study of the product distribution upon exposure of analog **10** to ferrous ions.[11-13] In contrast to the antimalarially active (IC_{50} = 4.0 ng/mL) benzyl ether of the $C_{4\beta}$-methyl analog **5** that reacted with ferrous bromide in THF to give a 1:4 ratio of a C_4-hydroxylated product like **2** and a ring-contracted product like **3**, the $C_{4\beta}$-phenyl analog **10** reacted under similar conditions to form ring-contracted acetal **11** as the only major product (eq 1); no more than a trace of any C_4-hydroxylated product like **2** was detectable. This result suggests that a $C_{4\beta}$-substituent that would make an adjacent carbon radical more stable than a tertiary radical in the upper pathway in Scheme 1 seems to shunt the ferrous ion reduction of that analog toward the lower pathway in Scheme 1, thereby actually avoiding formation of the C_4 radical intermediate that would lead to a C_4-hydroxylated product like **2**, characteristic of a potent antimalarial trioxane.



(1)

In conclusion, these results further support the importance of a carbon-centered radical leading to a C_4-hydroxylated product like **2** for high antimalarial activity of a 1,2,4-trioxane while also showing a limitation to this molecular mechanism; increasing the stability of such a radical beyond that of a simple tertiary radical by attaching a radical-stabilizing substituent[9] does not lead, as originally expected, to even higher antimalarial potency but rather to a partially or completely inactive analog.[2c] These structure–activity relationship generalizations[10] and an understanding of the mechanism[11,12] at the molecular level may help the design of better chemotherapeutic antimalarial trioxanes.

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(9) Approximate homolytic bond dissociation energies (BDE, kcal/mol) are as follows:

C–H	BDE
1°	98
2°	94.5
3°	91
$R_3SiCH_2CH_2$–H	88
$PhCH_2$–H	85

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