Antimalarial Cyclic Peroxy Ketals

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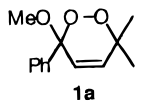
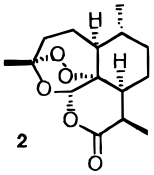
Over 20 new, cyclic, peroxy ketals have been prepared via a two-step protocol starting with readily available aryl methyl ketones. Structure−activity correlations using in vitro antimalarial data as a guide for optimization of potency have led to the design and synthesis of seven new peroxides that have IC_{50} values of 31−85 nM (artemisinin IC_{50} = 8.4 nM). Some SAR generalizations are discussed.

Because of the rapidly increasing threat worldwide of malaria epidemics resistant to alkaloid drugs such as chloroquine, there is an urgent global need to isolate from natural sources and/or to synthesize new classes of antimalarial compounds.[1−15] One natural and several synthetic endoperoxides have recently shown excellent efficacy as nonalkaloidal, fast-acting drugs effective for chemotherapy of malaria.[1−5] We recently reported the first example of antimalarial activity in a cyclic peroxy ketal (**1**) as well as a proposal for its mechanism of action.[16] Now we report in detail (1) preparation of over 20 such cyclic peroxy ketals, (2) structure−activity relationship (SAR) generalizations within this class of peroxides, and (3) identification of seven of these cyclic peroxy ketals that are between $^1/_4$ and $^1/_{10}$ as active in vitro against *Plasmodium falciparum* malaria parasites as the clinically used natural antimalarial cyclic peroxy ketal drug artemisinin (qinghaosu, **2**).



1a



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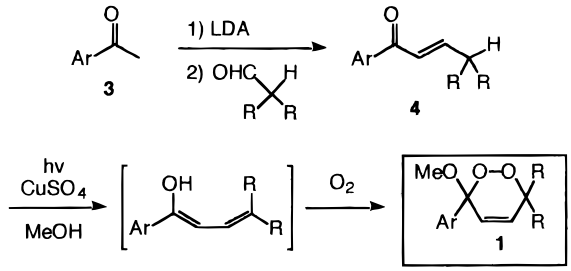
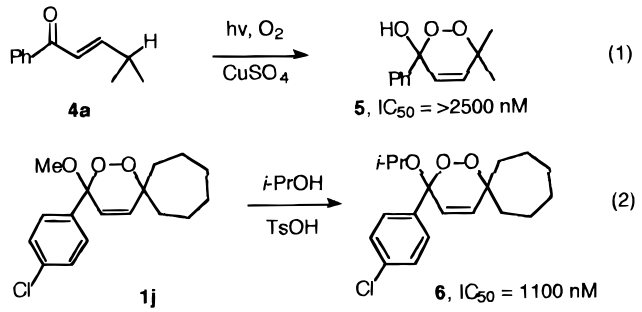
Chemistry

As shown in Scheme 1, enolization of a wide variety of readily available aryl methyl ketones **3** allowed aldol condensation with various α,α-disubstituted aldehydes to produce, after dehydration, the conjugated enones **4**. Then, Snider photoenolization and oxygenation[17,18] allowed direct preparation of unsaturated cyclic peroxy ketals **1**. The overall yields for this two-step procedure ranged from 16 to 61% (Table 2). Although the product peroxy ketals are stable compounds that appeared by TLC and NMR not to decompose when stored neat for several months at −20 °C, in vitro antimalarial testing of these samples did show diminished potency as a function of time. Slow diimide reduction[19] of unsaturated peroxide **1a** gave in low yield the corresponding saturated cyclic peroxy ketal that was expected to be

Scheme 1



inactive based on its inability (i.e. no olefinic bond) to form a critical *vicinal* diepoxide as required by the proposed mechanism of action for unsaturated peroxy ketal **1a**.[16] Subsequent in vitro antimalarial testing showed this saturated compound indeed to be inactive. Therefore, we studied the antimalarial potencies of only the unsaturated cyclic peroxy ketals shown in Scheme 1. When the Snider protocol was performed in the absence of methanol as solvent, cyclic peroxy hemiketal **5** was formed (eq 1) and was found to be antimalarially inactive. Other methods for synthesis of cyclic peroxy hemiketals are known.[20−23] When methoxy ketal **1j** was dissolved in isopropyl alcohol, acid catalyzed ketal exchange cleanly produced propoxy ketal **6** (eq 2) that was found to have little antimalarial activity, substantially lower than that of its precursor methoxy ketal **1j** (IC_{50} = 58 nM).



Biology

Following our previously described protocol for determining a new compound's in vitro antimalarial activity against *P. falciparum* malaria parasites,[24] we

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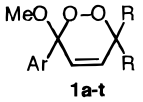
Table 1. [a] In Vitro Antimalarial Activities



1a-t

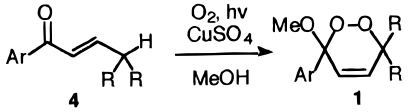
peroxy ketal	Ar	R, R	IC_{50}, nM	Scheme 1 overall yield, %
1a	Ph	Me, Me	1100	19
1b	Ph	cyclopentyl	190	24[b]
1c	Ph	cyclohexyl	280	20
1d	Ph	cycloheptyl	220	14
1e	4-MeOPh	cyclobutyl	160	6
1f	4-MeOPh	cyclohexyl	180	61
1g	4-MeOPh	cycloheptyl	210	29
1h	3,4,5-$(MeO)_3$Ph	cycloheptyl	120	13
1i	4-CF_3OPh	cycloheptyl	**61**	26
1j	4-ClPh	cycloheptyl	**58**	23
1k	4-FPh	cycloheptyl	**85**	31
1l	4-MeSPh	cycloheptyl	**78**	10
1m	4-MeS(O_2)Ph	cycloheptyl	**31**	100[c]
1n	4-EtPh	cycloheptyl	180	23
1o	4-MeSPh	cyclohexyl	160	34
1p	4-MeS(O_2)Ph	cyclohexyl	**56**	93[c]
1q	4-O_2NPh	cyclohexyl	**46**	4[d]
1r	4-ClPh	cyclohexyl	100	23
1s	4-FPh	cyclohexyl	200	24
1t	4-F_3CPh	cyclohexyl	140	16
artemisinin (**2**)			8.4 ± 1.2	

[a] Antimalarial activity was determined as reported previously.[24] The standard deviation for each set of quadruplicates was an average of 9% (≤38%) of the mean. R^2 values for the fitted curves were ≥0.979. Artemisinin activity is mean ± standard deviation of concurrent control (n = 20). [b] Prepared in three steps from 3-cyclopentylpropionyl chloride. [c] Prepared by *m*-CPBA oxidation of the corresponding sulfide. [d] Photoenolization−oxygenation step yielded the hemiketal, which was subsequently converted into the methoxy ketal by acid-catalyzed exchange.

evaluated the 20 peroxy ketals **1a**−**t** (Table 1). This in vitro assay actually was crucial in providing structure−activity relationship (SAR) correlations after only a few of these peroxy ketals were made so that design of the next members of the series could be modified to optimize antimalarial activity; this iterative process of synthesis and then biological testing provided critical SAR feedback that greatly facilitated progress toward preparation of potent peroxy ketals.

The first structural parameter in peroxy ketal **1** that was explored in depth involved the R groups. Whereas dimethyl peroxide **1a** had an IC_{50} of 1100 nM, the corresponding carbocyclic peroxides **1b**−**d** had IC_{50} values below 300 nM. No large difference in potency was evident as the carbocyclic ring was increased in size from 5 (**1b**) to 6 (**1c**) to 7 (**1d**). Likewise, in *p*-methoxyphenyl carbocyclic peroxides **1e**−**g**, antimalarial activity did not change appreciably in going from carbocyclic ring size 4 (**1e**) to 6 (**1f**) to 7 (**1g**). The second structural parameter investigated in depth involved changing the substituents on the aryl group of peroxy ketal **1**. In comparison to *p*-methoxyphenyl cycloheptyl peroxide **1g** (IC_{50} = 210 nM), trimethoxyphenyl cycloheptyl peroxide **1h** was more potent (IC_{50} = 120 nM). Even more so, *p*-trifluoromethoxy cycloheptyl peroxide **1i** (IC_{50} = 61 nM) was about $^1/_7$ as potent as artemisinin (**2**). Halogen substitutents on the aromatic ring also were advantageous. Thus, *p*-chlorophenyl cycloheptyl peroxide **1j** had an IC_{50} of 58 nM, and the corresponding *p*-fluorophenyl peroxide **1k** had an

Table 2. Summary of Experimental Results (Scheme 1)



4 **1**

4, mg (% yield)	**1**, mg (% yield)	physical state (mp, °C)	HPLC t_R, min (solvent, % EtOAc/hexane)
4a, 700 (50)	**1a**, 119 (38)	solid (35−38)	9.9 (10)
4b, 287 (47)[a]	**1b**, 144 (51)	oil	8.8 (10)
4c, 640 (39)	**1c**, 150 (52)	solid (33−35)	8.3 (10)
4d, 114 (26)	**1d**, 49 (53)	oil	8.0 (10)
4e, 640 (40)	**1e**, 20 (16)	oil	9.1 (20)
4f, 980 (100)	**1f**, 227 (61)	oil	12.3 (10)
4g, 220 (48)	**1g**, 76 (60)	oil	8.0 (20)
4h, 285 (50)	**1h**, 41 (25)	solid (98−100)	15.7 (20)
4i, 340 (61)	**1i**, 51 (43)	solid (76−78)	8.1 (10)
4j, 192 (41)	**1j**, 83 (55)	solid (71−73)	8.1 (10)
4k, 210 (47)	**2k**, 88 (66)	solid (48−49)	8.5 (10)
4l, 137 (23)	**1l**, 59 (43)	solid (68−70)	10.3 (10)
	1m, 39 (100)[b]	oil	9.2 (50)
4n, 220 (48)	**1n**, 62 (47)	oil	7.3 (10)
4o, 940 (100)	**1o**, 110 (34)	oil	10.7 (10)
	1p, 31 (93)[b]	oil	9.5 (50)
4q, 106 (13)	**1q**, 42 (33)[c]	solid (77−79)	8.6 (20)
4r, 390 (44)	**1r**, 71 (53)	solid (83−85)	8.5 (10)
4s, 430 (38)	**1s**, 186 (64)	solid (55−57)	8.7 (10)
4t, 234 (46)	**1t**, 46 (35)	solid (60−62)	9.3 (10)

[a] Prepared in two steps from 3-cyclopentylpropionyl chloride, overall yield. [b] Prepared by *m*-CPBA oxidation of the corresponding sulfide. [c] Photoenolization−oxygenation step yielded the hemiketal, which was subsequently converted into the methoxy ketal by acid-catalyzed exchange, overall yield.

IC_{50} of 85 nM. Theinductively electron-withdrawing ***p*-sulfone** substituent imparted higher antimalarial potency than did the corresponding ***p*-sulfide** substituent (in Table 1, compare peroxides **1m** vs **1l** and **1p** vs **1o**). Other recent examples show also that sulfones are more potent antimalarials than the corresponding sulfides.[25,26] Finally, peroxide **1q,** carrying also a strongly electron-withdrawing *p*-nitro group on the aromatic ring, was very potent.

Despite the earlier conclusion that carbocyclic ring size did not appreciably alter potency in phenyl peroxides **1b**−**d** and in *p*-methoxyphenyl peroxides **1e**−**g**, *p*-chlorophenyl cyclo**hexyl** peroxide **1r** (IC_{50} = 100 nM) was found to be considerably less potent than *p*-chlorophenyl cyclo**heptyl** peroxide **1j** (IC_{50} = 58 nM). Likewise, *p*-fluorophenyl cyclo**hexyl** peroxide **1s** (IC_{50} = 200 nM) was found to be considerably less potent than *p*-fluorophenyl cyclo**heptyl** peroxide **1k** (IC_{50} = 85 nM). Also in the sulfide/sulfone set of four compounds, the cyclo**hexyl** carbocycles **1o** and **1p** were found to be much less potent than the corresponding cyclo**heptyl** carbocycles **1l** and **1m**. We do not yet have a good rationale for this SAR trend.

In conclusion, regular feedback from in vitro antimalarial testing has allowed iterative design and synthesis of several potent cyclic peroxy ketals. Of the 20 such easily synthesized peroxides reported in Table 1, seven (**1i**−**m**, **1p**, and **1q**) have antimalarial potencies between $^1/_4$ to $^1/_{10}$ that of the clinically used natural trioxane antimalarial artemisinin (**2**). Thus, further biological evaluation of these seven new, readily prepared peroxides will establish ultimately whether they will become promising drug candidates in the international effort using chemotherapy to combat malaria.

Experimental Section

General. Unless otherwise noted, reactions were run in oven-dried glassware under an atmosphere of argon. Diethyl ether (ether) and tetrahydrofuran (THF) were distilled from sodium benzophenone ketyl prior to use. Methylene chloride (CH_2Cl_2) was distilled from calcium hydride prior to use. All other compounds were purchased from Aldrich Chemical Co. and used without further purification. Analytical thin-layer chromatography (TLC) was conducted with silica gel 60 F_{254} plates (250 mm thickness, Merck). Column chromatography was performed using flash silica gel (partical size 400−230 mesh). Yields are not optimized. Purity of final products was judged to be >95% based on their chromatographic homogeneity. High-performance liquid chromatography (HPLC) was carried out with a Rainin HPLX system equipped with two 25 mL/min preparative pump heads using a Rainin Dynamax 10 mm × 250 mm (semipreparative) column packed with 60 Å silica gel (8 μm pore size) as bare silica. Melting points were measured using a Mel-Temp metal-block apparatus and are uncorrected. Nuclear magnetic resonance (NMR) spectra were obtained either on a Varian XL-400 spectrometer, operating at 400 MHz for ^{1}H and 100 MHz for ^{13}C. Chemical shifts are reported in parts per million (ppm, δ) downfield from tetramethylsilane. Splitting patterns are described as singlet (s), doublet (d), triplet (t), quartet (q), multiplet (m), and broad (br). Infrared (IR) spectra were obtained using a Perkin-Elmer 1600 FT-IR spectrometer. Resonances are reported in wavenumbers (cm^{-1}). Low- and high-resolution mass spectra (LRMS and HRMS) were obtained with electronic or chemical ioniztion (EI or CI) either (1) at Johns Hopkins University on a VG Instruments 70-S spectrometer run at 70 eV for EI and run with ammonia (NH_3), butane (C_4H_{10}), or methane (CH_4) as carrier gas for CI or (2) at the University of Illinois at Champaign−Urbana on a Finnigan-MAT CH5, a Finnigan-MAT 731, or a VG Instruments 70-VSE spectrometer run at 70 eV for EI and run with methane (CH_4) for CI. Combustion analyses were conducted by Atlantic Microlab (Norcross, GA).

General Procedure 1: Enone Synthesis (Aldol Condensation, Dehydration). An LDA solution (1.1 equiv based on 1.0 equiv of acetophenone substrate) was prepared by treating diisopropylamine (1.1 equiv) in THF (volume needed to make final concentration of LDA 0.3−0.5 M) at −78 °C with *n*-BuLi (1.7 M solution in hexanes, 1.1 equiv). The LDA solution was stirred at −78 °C for 10 min and then at room temperature for 10 min, then re-cooled to −78 °C. Acetophenone (1.0 equiv, either neat in the case of liquids or as a 0.5−1.0 M solution in THF) was added to the LDA solution and stirred at −78 °C for 15 min and then at room temperature for 10 min. The reaction was cooled to −78 °C, and aldehyde (1.0−1.2 equiv) was added. The reaction was stirred at −78 °C for 1 h and then at room temperature for 1 h. The reaction was quenched (saturated aqueous NH_4Cl), extracted (ether), washed (H_2O, brine), dried (Na_2SO_4), and concentrated under reduced pressure to give crude product.

General Procedure 2: Peroxy Ketal Synthesis (Photoenolization, Oxygenation). In a 125 mL three-necked sulfonation flask equipped with a screw-cap, oxygen inlet, and a 13 cm Vigreux distilling column with a gas outlet to a mineral oil bubbler, a solution of enone (75−350 mg) and $CuSO_4$ (15−40 mg) in CH_2Cl_2 (100 mL) and MeOH (10 mL) was irradiated at room temperature by a 275 W sun lamp (placed ca. 10 cm from reaction vessel) while ultrahigh purity grade oxygen was bubbled through the solution at a rate of 20 mL/min. The flask was cooled by a fan, and solvent was refilled as required. After 14−17 h the reaction was stopped, washed (water), dried (Na_2SO_4), and concentrated to give crude product.

Dimethyl Phenyl Enone 4a. Acetophenone (0.93 mL, 8.0 mmol) was treated with LDA (9.0 mmol) followed by isobutyraldehyde (0.91 mL, 10 mmol) according to general procedure 1. Flash column chromatography of the crude product using 5% EtOAc/hexanes on silica gel gave desired enone **4a** (0.70 g, 4.0 mmol, 50%) as an oil: ^{1}H NMR ($CDCl_3$) δ 1.13 (d, 6 H, J = 6.4), 2.6 (m, 1 H), 6.82 (dd, 1 H, J = 1.6, 15.6), 7.04 (dd, 1 H, J = 6.8, 15.6 Hz), 7.6−7.4 (m, 3 H), 7.95−7.90 (m, 2 H); ^{13}C NMR ($CDCl_3$) δ 21.4, 31.5, 123.0, 128.41, 128.44, 132.5, 138.0, 156.0, 191.3; FT-IR (neat, cm^{-1}) 1672, 1619.

Dimethyl Phenyl Peroxy Ketal 1a. Phenyl enone **4a** (247 mg, 1.42 mmol) and copper(II) sulfate (20 mg) were irradiated for 17 h according to general procedure 2. Flash column chromatography of the crude product using 5% EtOAc/hexanes on silica gel gave desired peroxide **1a** (119 mg, 0.54 mmol, 38%) as a white solid: mp 35−38 °C; ^{1}H NMR ($CDCl_3$) δ 1.33 (s, 3), 3.38 (s, 3), 5.78 (d, 1, J = 10 Hz), 5.98 (d, 1, J = 10 Hz), 7.4−7.3 (m, 3), 7.5 (m, 2); ^{13}C NMR ($CDCl_3$) δ 24.53, 24.60, 51.4, 76.9, 99.6, 125.7, 126.4, 128.5, 128.6, 134.1, 138.0; HRMS *m/z* ($M + H^+$) calcd. 221.1178, found 221.1179. Ketal **1a** was further purified using HPLC prior to antimalarial testing (10% EtOAc/hexanes, 3.0 mL/min, 254 nm, t_R = 9.9 min).

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Supporting Information Available: ^{1}H and ^{13}C NMR, IR, and mass spectral data for peroxy ketals **1b**−**t** and for hemiketal **5** and propoxy ketal **6** (8 pages). Ordering information is given on any current masthead page.

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