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Research Article

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Synthesis and Antimalarial Activity of Dihydroperoxides and Tetraoxanes Conjugated with Bis(benzyl)acetone Derivatives

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Dihydroperoxides and tetraoxanes derived from symmetrically substituted bis(arylmethyl)acetones were synthesized in modest to good yields using several methods. Three of these compounds exhibit an important *in vitro* antimalarial activity ($1.0\ \mu M \leq IC_{50} \leq 5.0\ \mu M$) against blood forms of the human malaria parasite *Plasmodium falciparum*.

Key words: antiplasmodial, antiprotozoal, malaria, *Plasmodium falciparum*

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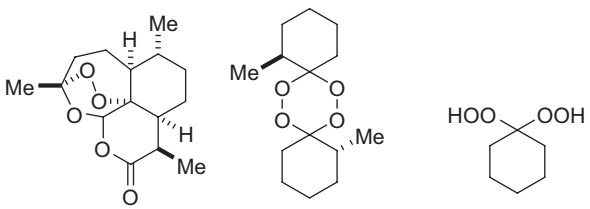
Many compounds having a peroxide functional group exhibit antimalarial activity as exemplified by the natural product artemisinin and its semi-synthetic derivatives (Figure 1) (1). Artemisinin-based antimalarials are highly potent, and little or no cross-resistance has been observed for this class of compound that contains a unique 1,2,4-trioxane heterocycle as pharmacophore. Although artemisinin produces an initially rapid clearance of parasites from human blood, its clinical utility has been limited by early recrudescence and low oral activity (2–4). These limitations have stimulated the development of antimalarials, which are structurally less complex than artemisinin such as the tetraoxanes. The latter have two endoperoxide bonds and are inexpensive, easy to synthesize, and highly active antimalarial agents (5) as illustrated by di-*spiro*-1,2,4,5-tetraoxane WR 148999 (Figure 1) (6). An interesting way to synthesize tetraoxanes is via dihydroperoxides that are known key intermediates in the synthesis of many classes of peroxides, many of which have antimalarial activity (Figure 1).

Curcumin is an α,β-unsaturated ketone, which is obtained from the roots of *Curcuma longa* (Figure 2) (7). It has proven antimalarial activity *in vitro* (IC_{50} = 5 μM) against blood forms of chloroquine-resistant *Plasmodium falciparum* and is also active *in vivo* against *Plasmodium berghei* when administered orally in mice (8). Curcumin also has significant antitumor activity, and bis(arylidene)acetones were introduced as curcumin analogs having improved solubility and other properties (9,10). Furthermore, a series of ring-substituted 1,5-bis-(phenyl)pentadien-3-ones have been prepared as curcumin analogs and been patented for their anti-*Plasmodium* and anti-*Trypanosoma* properties (11).

In this context, we report herein the synthesis of dihydroperoxides and tetraoxanes having bis-(substituted)phenylethyl substructural units. Based on the antimalarial activity of curcumin and patented 1,5-bis-(phenyl)pentadien-3-ones and of the (endo)peroxide moiety, these compounds were believed to have potential as antimalarial agents (Scheme 1).

Methods and Materials

Melting points were measured in a Microquímica MQAPF melting point apparatus and are uncorrected. Infrared spectra were recorded



Artemisinin **WR_{148999}** **A Dihydroperoxide**

Figure 1: Structures of O–O bond containing antimalarials and 1,1-dihydroperoxycyclohexane.

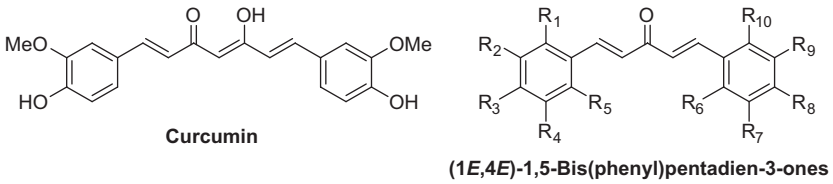
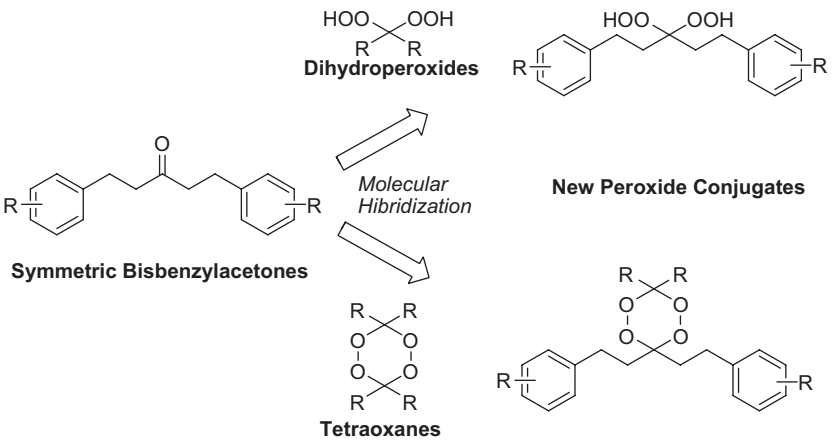


Figure 2: Antimalarial natural product curcumin and patented synthetic analogs.



Scheme 1: Reactions between dihydroperoxides and tetraoxanes and bisbenzylacetones.

with a BOMEM-FTIR MB102 spectrometer. Ultraviolet (UV) spectra were recorded with a UV-1601 PC Shimadzu. ^{1}H and ^{13}C NMR spectra were recorded in deuterochloroform ($CDCl_3$) with a Bruker Avance DRX300 (300 MHz) spectrometer. Chemical shifts (δ) were reported in parts per million (ppm) with reference to tetramethylsilane (TMS) as internal standard. The following abbreviations were used for the ^{1}H multiplicities: singlet (s), doublet (d), triplet (t), and multiplet (m). Coupling constants (*J*) were reported in Hertz (Hz). Thin-layer chromatography (TLC) was performed on silica gel supported on glass plates (Silica Gel F254; Merck) with a fluorescent indicator and visualized under a UV lamp (254 nm) and/or in I_2 vapor and/or with a solution of 1% anisaldehyde with H_2SO_4 (1% v/v) in EtOH. Column chromatography was performed using silica gel 60G (63–200 μm, 70–230 mesh ASTM; Merck, Waltham, MA, USA).

General procedure for the preparation of dibenzalacetones 4–6

A solution of aldehyde (30 mmol) in EtOH (10 mL) was added to a solution of acetone (25 mmol) and NaOH (3 g, 75 mmol) in EtOH (10 mL) at 0 °C. The reaction mixture was stirred for 15 min and allowed to come to room temperature. The precipitate was filtered, washed with a cold mixture of EtOH/H_2O, and recrystallized from a mixture of hexane/EtOAc to yield dibenzalacetone derivatives **4–6**.

General procedure for the synthesis of pentan-3-one derivatives 7–9

To a solution of dibenzalacetone **4–6** (10 mmol) in EtOAc (30 mL) was added 10% Pd/C (30 mg, 0.1 mmol). The suspension was hydrogenated for 6 h at 4 psi for 12–24 h. The catalyst was removed by filtration, and the resulting solution was evaporated under reduced pressure. The residue was purified by chromatography on silica gel (hexane/EtOAc 8:2) to give pentan-3-ones **7–9**.

Representative synthesis of dihydroperoxides using 30% H_2O_2 and I_2 in MeCN

I_2 (0.23 g, 1 mmol) and 30% H_2O_2 (1.36 g, 40 mmol) were added to a solution of ketone **10** or **7** (10 mmol) in MeCN (30 mL). The reaction mixture was stirred at room temperature for 24 h (72 h when dibenzalacetone derivatives were used as starting materials) and was then diluted with CH_2Cl_2 (10 mL) and H_2O (20 mL). The organic layer was successively washed with saturated aqueous NaCl, dried with Na_2SO_4, and filtered. The solvents were evaporated to dryness under reduced pressure, and the resulting residue was purified by column chromatography on silica gel (hexane/EtOAc 8:2) to afford dihydroperoxides **11** or **13**, respectively.

MTO in TFE procedure

H_2O_2 30% (1.36 g, 40 mmol) and methyltrioxorhenium (MTO) (0.0003 g, 0.001 mmol) were added to a solution of ketone **10** or **7** (10 mmol) in trifluoroethanol (15 mL). The reaction mixture was stirred at room temperature for 24 h and was then diluted with CH_2Cl_2 (10 mL) and H_2O (20 mL). The organic layer was washed with saturated aqueous NaCl, dried with Na_2SO_4, and filtered. The solvents were evaporated to dryness under reduced pressure, and the resulting residue was purified by column chromatography on silica gel (hexane/EtOAc 9:1) to afford dihydroperoxide **11** and dimer **12** or dihydroperoxide **13** and dimer **16**, respectively.

HCl in CH_2Cl_2/MeCN procedure

H_2O_2 30% (3.4 g, 100 mmol) and concentrated HCl (six drops) were added to a solution of ketone **10** or **7** (10 mmol) in a mixture of CH_2Cl_2 (10 mL) and MeCN (30 mL). The reaction mixture was stirred at room temperature for 24 h and was then diluted with CH_2Cl_2 (10 mL) and water (20 mL). The organic layer was successively washed with saturated aqueous $NaHCO_3$ (30 mL), water (30 mL), and saturated aqueous NaCl. The organic phase was dried with Na_2SO_4 and filtered. The solvents were removed under reduced pressure, and the resulting residue was purified by column chromatography on silica gel (hexane/EtOAc 9:1) to afford dihydroperoxides **11** or **13**.

H_2O_2 in ether procedure

Preparation of H_2O_2 in ether (12). Thirty percent H_2O_2 solution (50 mL) was saturated with NaCl, and the mixture was stirred at room temperature for 10 min. After the initial cloudy solution became clear, it was extracted with Et_2O (3 × 30 mL). The organic phase was separated and dried with Na_2SO_4, filtered and used without purification. Ketones **7–10** (10 mmol) were dissolved in the freshly prepared solution of H_2O_2 in Et_2O (30 mL) described above, and phosphomolybdic acid (10 mg) was added. The reaction mixture was stirred at room temperature for 24–72 h and was then diluted with EtOAc (10 mL) and water (20 mL). The organic layer was washed with saturated aqueous NaCl, dried with Na_2SO_4, and filtered. The solvents were evaporated to dryness under reduced pressure, and the resulting residue was purified by column chromatography on silica gel (EtOAc/hexane, 2:1) to afford dihydroperoxides **11, 13**, **14**, and **15**.

Representative synthesis of tetraoxanes using 30% H_2O_2 and H_2SO_4, EtOH, H_2O

Ketone **7** (10 mmol) and 30% H_2O_2 (0.34 g, 10 mmol) were added to a solution of concentrated H_2SO_4 (20 mL) in EtOH (15 mL) and H_2O (15 mL). The reaction mixture was stirred at 0 °C for 14 h. The precipitated material was filtered and recrystallized in MeCN furnishing the tetraoxane **17** (0.20 g; 0.8 mmol, 8% yield).

HBF_4 in TFE

Dihydroperoxide **13** (2.88 g, 10 mmol) and 30% H_2O_2 (0.34 g, 10 mmol) were added to a solution of ketone **7** or cyclohexanone (10 mmol) in trifluoroethanol (15 mL). The reaction mixture was stirred at room temperature for 24 h and was then diluted with CH_2Cl_2 (10 mL) and water (20 mL). The organic layer was washed with saturated aqueous NaCl, dried with Na_2SO_4, and filtered. The solvents were evaporated to dryness under reduced pressure, and the resulting residue was purified by column chromatography on silica gel (hexane/EtOAc 9:1) to afford compound **17** or **18** as a white solid in 22 or 38% yield, respectively

MTO/HBF_4 in TFE

30% H_2O_2 (0.07 g, 2 mmol) and methyltrioxorhenium (0.0003 g, 0.001 mmol) were added to a solution of cyclohexanone (1 mmol) in trifluoroethanol (2 mL). The reaction mixture was stirred for 3 h at room temperature; then, ketone **4** or **7** (1 mmol) and HBF_4 were added (0.08 g, 1 mmol). After stirring for 24 h at room temperature, the reaction was diluted with CH_2Cl_2 (1 mL) and H_2O (2 mL). The organic layer was washed with saturated aqueous NaCl, dried with Na_2SO_4, and filtered. The solvents were evaporated to dryness under reduced pressure, and the resulting residue was purified by column chromatography on silica gel (hexane/EtOAc 9:1) to afford compounds **18** or **19** in 28 or 18% yield, respectively.

Data

(1*E*,4*E*)-1,5-Bis(phenyl)-penta-1,4-dien-3-one (**4**): Yellow crystals, mp 109.9–110.6 °C (lit. (13)): 111.0–113.0 °C); Yield: 95%; IR (ν, /cm): 3054, 3026, 1651, 1627, 1592, 1496, 1443, 1343, 1195, 983, 762, 696; UV (λ, nm): 326.0; ^{1}H NMR (300 MHz, $CDCl_3$, ppm): δ 7.10 (d, J = 13.0 Hz, 2H, H2, H4), 7.40–7.63 (m, 10H, H arom), 7.75 (d, J = 13.0 Hz, 2H, H1, H5); ^{13}C NMR (75 MHz, $CDCl_3$, ppm): δ 125.4 (C2, C4), 128.4 (C arom), 128.9, 130.4, 134.8 (CH arom), 143.3 (C1, C5), 188.6 (C=O).

(1*E*,4*E*)-1,5-Bis(4-methoxyphenyl)penta-1,4-dien-3-one (**5**): Yellow crystals, mp 129.3–129.8 °C; Yield: 91% IR (ν, /cm): 2961, 2841, 1653, 1630, 1597, 1572, 1421, 1251, 1178, 1031, 981, 830; UV (λ, nm): 361.0; ^{1}H NMR (300 MHz, $CDCl_3$, ppm): δ 3.82 (s, 6H, 2 × OCH_3), 6.90 (d, J = 8.7 Hz, 4H, H arom), 6.94 (d, J = 15.9 Hz, 2H, H2, H4), 7.54 (d, J = 8.7 Hz, 4H, H arom), 7.68 (d, J = 15.9 Hz, 2H, (H1, H5); ^{13}C NMR (75 MHz, $CDCl_3$, ppm): δ 55.3 (2 × OCH_3), 114.4 (CH arom), 123.5 (C2, C4), 127.6 (C arom), 130.0 (CH arom), 142.5 (C1, C5), 161.5 (C arom), 188.7 (C=O).

(1*E*,4*E*)-1,5-Bis(3,4,5-trimethoxyphenyl)penta-1,4-dien-3-one (**6**): Yellow crystals, mp 130.1–131.4 °C; Yield: 76% IR (ν, /cm): 3015, 3030, 2818, 2842, 1626; ^{1}H NMR (300 MHz, $CDCl_3$, ppm): δ 3.91 (s, 18H, 6 × OCH_3); 6.94 (s, 4H, H arom); 7.00 (d, J = 12.0 Hz, 2H, H2, H4), 7.67 (d, J = 12.0 Hz, 2H, H1, H5); ^{13}C NMR (75 MHz, $CDCl_3$, ppm): δ 56.5 (6 × OCH_3), 105.4 (4 × CH arom), 125.0 (C2, C4), 130.4 (C arom), 145.6 (3 × C arom), 155.7 (C1, C5), 188.6 (C=O).

1,5-Bis(phenyl)pentan-3-one (**7**): Yellow oil; Yield: 95%; IR (ν, /cm): 3109, 3000, 2960, 2855, 1713; ^{1}H NMR (300 MHz, $CDCl_3$, ppm): δ 2.67 (t, J = 7.5 Hz, 4H, H1, H1′, H5, H5′); 2.88 (t, J = 7.5 Hz, 4H, H2, H2′, H4, H4′), 7.14 (d, J = 7.0 Hz, 4H, H arom); 7.27 (m, J = 7.0 Hz, 6H, H arom); ^{13}C NMR (75 MHz, $CDCl_3$, ppm): δ 29.7 (C1, C5), 44.4 (C2, C4), 126.4, 129.2 (CH arom), 141.0 (C arom), 208.9 (C=O).

1,5-Bis(4-methoxyphenyl)pentan-3-one (**8**): White solid, mp 53.2–54.5 °C [lit. (14) mp 55.0–55.2 °C]; Yield: 78%; IR (ν, /cm): 3109, 3033, 2830, 2905, 1705. ^{1}H NMR (300 MHz, $CDCl_3$, ppm): δ 2.65 (t, J = 7.5 Hz, 4H, H1, H1′, H5, H5′), 2.81 (t, J = 7.5 Hz, 4H, H2, H2′, H4, H4′), 3.76 (s, 6H, 2 × OCH_3), 6.79 (d, J = 8.5 Hz, 4H, H arom), 7.05 (d, J = 8.5 Hz, 4H, H arom); ^{13}C NMR (75 MHz, $CDCl_3$, ppm): δ 29.0 (C1, C5), 44.9 (C2, C4), 55.4 (2 × OCH_3), 114.0, 129.4 (CH arom), 133.2 (C arom), 158.1 (C arom), 209.6 (C=O).

1,5-Bis(3,4,5-trimethoxyphenyl)pentan-3-one (**9**): White solid, mp 53.2–54.5 °C; Yield: 51%; IR (ν, /cm): 3010, 3025, 2920, 1700; ^{1}H NMR (300 MHz, $CDCl_3$, ppm): δ 2.74 (t, J = 7.0 Hz, 4H, H2, H2′,

H4, H4′), 2.81 (t, J = 7.0 Hz, 4H, H1, H1′, H5, H5′), 3.81 (s, 18H, 6 × OCH_3), 6.38 (s, 4H, H arom). ^{13}C NMR (75 MHz, $CDCl_3$, ppm): δ 30.2 (C1, C5), 44.8 (C2, C4), 56.1 (6 × OCH_3), 105.3 (CH arom), 136.9 (C arom), 153.3 (C arom), 209.1 (C=O).

4-*tert*-Butyl-1,1-dihydroperoxycyclohexane (**11**): Yield: 89%[1]; H NMR (300 MHz, $CDCl_3$, ppm): δ 0.87 (s, 14H, 3 × CH_3, H3, H4), 1.35 (m, 2H, H2, Ha, Ha'), 2.26 (m, 2H, H2, Hb, Hb'), 8.10 (sl, 2H, H7); ^{13}C NMR (75 MHz, $CDCl_3$, ppm): δ 23.5 (C2), 27.7 (C6), 29.9 (C2), 41.5 (C5), 47.6 (C4), 110.5 (C1).

1,1′-Peroxybis(4-*tert*-butyl(hydroperoxy)cyclohexane) (**12**): White solid, mp 80.9–81.3 °C; Yield: 41%; IR (ν, ⁄cm): 3411, 3428 (O-O-H), 2891, 2910; ^1H NMR (300 MHz, $CDCl_3$, ppm): δ 1.05 (s, 18H, 6 × CH_3); 1.26 (m, 2H, 2 × H4), 1.49 (m, 8H, 2 × H2, H2′, H6, H6′), 2.32 (m, 8H, 2 × H3, H3′, H5, H5′), 9.62 (s, 2H, 2 × OOH); ^{13}C NMR (75 MHz, $CDCl_3$, ppm): δ 23.2 (C3), 23.5 (C2), 27.8 (9 × CH_3), 30.1 ($\underline{C}(CH_3)_3$), 47.6 (C4), 111.3 (C1).

3,3-Dihydroperoxy-1,5-(bisphenyl)pentane (**13**): White solid, mp 85.4–86.7 °C; Yield: 84%; IR (ν, ⁄cm): 3278, 3415 (O-O-H), 3024, 3091, 2888, 2940; ^1H NMR (300 MHz, $CDCl_3$, ppm): δ 2.07 (t, J = 8.3 Hz, 4H, H1, H1′, H5, H5′), 2.73 (t, J = 8.0 Hz, 4H, H2, H2′, H4, H4′), 7.22 (m, 10H, H arom), 9.28 (s, 2H, OOH); ^{13}C NMR (75 MHz, $CDCl_3$, ppm): δ 30.1 (C2, C4), 31.3 (C1, C5), 113.9 (C3), 126.4, 128.7 (CH arom), 141.3 (C arom).

3,3-Dihydroperoxy-1,5-bis(4′-methoxyphenyl)pentane (**14**): White solid, mp 170.2–173.1 °C; Yield: 50%; IR (ν, ⁄cm): 3435, 3250 (O-O-H), 2885, 2720; ^1H NMR (300 MHz, $CDCl_3$, ppm): δ 1.25 (t, J = 6.5 Hz, 4H, H1, H1′, H5, H5′), 2.05 (t, J = 6.5 Hz, 4H, H2, H2′, H4, H4′), 3.72 (s, 6H, 2 × OCH_3), 6.81 (d, J = 8.5 Hz, 4H, H arom), 7.08 (d, J = 8.5 Hz, 4H, H arom), 9.78 (large s, 2H, OOH); ^{13}C NMR (75 MHz, $CDCl_3$, ppm): δ 29.1 (C2, C4), 31.6 (C1, C5), 113.4 (C3), 114.1, 129.4 (CH arom), 133.6 (C arom), 158.0 (2 × OCH_3).

3,3-Dihydroperoxy-1,5-bis(3′,4′,5′-trimethoxyphenyl)pentane (**15**): Yellow oil; Yield: 31%; IR (ν, ⁄cm): 3125, 3319 (O-O-H), 2884, 2920, 2762, 2809. ^1H NMR (300 MHz, $CDCl_3$, ppm): δ 2.06 (t, J = 7.0 Hz, 4H, H2, H2′, H4, H4′), 2.72 (t, J = 7.0 Hz, 4H, H1, H1′, H5, H5′), 3.84 (s, 18H, 6 × OCH_3), 6.43 (s, 4H, H arom), 9.50 (s, 2H, OOH); ^{13}C NMR (75 MHz, $CDCl_3$, ppm): δ 30.6 (C2, C4), 31.5 (C1, C5), 56.3, 61.2 (6 × OCH_3), 105.6 (CH arom), 112.4 (CH arom), 133.6 (C arom), 153.3 (OCH_3).

[3,3′-Peroxybis-(3-hydroperoxypentane-5,3,1-triyl)]tetrabenzene (**16**): White solid, mp 140.9–141.3 °C; Yield: 23%; IR (ν, ⁄cm): 3370, 3458 (O-O-H), 2916, 3021, 2991, 2844; ^1H NMR (300 MHz, $CDCl_3$, ppm): δ 2.11 (d, J = 8.0 Hz, 8H, 2 × (H1, H1′, H5, H5′)), 2.78 (d, J = 8.0 Hz, 8H, 2 × (H2, H2′, H4, H4′)), 7.18 (m, 4H, H arom), 7.30 (m, 16H, H arom), 9.70 (s, 2H, 2 × OOH); ^{13}C NMR (75 MHz, $CDCl_3$, ppm): δ 30.3 (2 × C1, C5), 31.8 (2 × C2, C4), 114.3 (C3), 126.6 (4 × CH arom), 128.7 (16 × CH arom), 141.2 (4 × C arom).

3,3,6,6-Tetraphenethyl-1,2,4,5-tetraoxane (**17**): White solid, mp 149.7–150.9 °C; Yield: 22%; IR (ν, ⁄cm): 3075, 3244 (O-O-H), 2990, 3966, 2830, 2974; ^1H NMR (300 MHz, $CDCl_3$, ppm): δ 2.04 (m, 8H, 4 × $PhC\underline{H}_2CH_2$), 2.72 (m, 8H, 4 × $PhCH_2C\underline{H}_2$), 7.27 (m, 2OH, H arom); ^{13}C NMR (75 MHz, $CDCl_3$, ppm): δ 26.6 ($Ph\underline{C}H_2CH_2$), 36.2 ($PhCH_2\underline{C}H_2$), 110.2 (C3, C6), 141.9 (C arom), 125.4, 127.6, 129.7 (CH arom).

3,3-Diphenethyl-1,2,4,5-tetraoxaspiro[5.5]undecane (**18**): White solid, mp 114.5–115.3 °C; Yield: 38%; IR (ν, ⁄cm): 3224 (O-O-H), 3117, 3008, 2902, 2814, 2775; ^1H NMR (300 MHz, $CDCl_3$, ppm): δ 1.47 (s, 10H, cyclohexyl), 2.28 (m, 8H, 2 × $(CH_2)_2Ph$), 7.20–7.30 (m, 10H, H arom); ^{13}C NMR (75 MHz, $CDCl_3$, ppm): δ 21.6 ($CH_2\underline{C}H_2CH_2\underline{C}H_2CH_2$), 25.6 ($CH_2\underline{C}H_2Ph$), 32.1 ($\underline{C}H_2CH_2CH_2\underline{C}H_2CH_2$, $\underline{C}H_2CH_2Ph$), 110.1 (C3), 126.2 (CH arom), 128.2 (C arom), 128.6 (C arom).

3,3-Bis(3-phenyloxiran-2-yl)-1,2,4,5-tetraoxaspiro[5.5]undecane (**19**): White solid, 87.5–89.1°C; Yield: 18%; IR (ν, ⁄cm): 3012, 3184 (O-O-H), 2992, 3002, 2765, 2916; ^1H NMR (300 MHz, $CDCl_3$, ppm): δ 1.26 (m, 2H, $CH_2CH_2\underline{C}H_2CH_2CH_2$), 1.31 (m, 6H, $CH_2\underline{C}H_2CH_2\underline{C}H_2CH_2$), 1.39 (m, 4H, $\underline{C}H_2CH_2CH_2CH_2\underline{C}H_2$), 4.13 (m, 1H, ), 4.37 (m, 1H,), 7.26 (d, J = 7.0 Hz, 2H, H arom), 7.72 (d, J = 7.0 Hz, 4H, H arom), 8.06 (d, J = 7.0 Hz, 4H, H arom); ^{13}C NMR (75 MHz, $CDCl_3$, ppm): δ 14.5 ($CH_2CH_2\underline{C}H_2CH_2CH_2$), 29.9 ($CH_2\underline{C}H_2CH_2\underline{C}H_2CH_2$), 41.4 ($CH_2\underline{C}H_2CH_2\underline{C}H_2CH_2$), 60.7 (), 61.1 (), 118.5 (C3, C6), 128.2 (CH arom), 130.4 (CH arom), 167.2 (C arom).

Antimalarial activity

In vitro culture of human malaria parasite *Plasmodium falciparum*

Chloroquine-resistant *P. falciparum* K1 strain was obtained from the Malaria Research Reference Reagent Resource Center (MR4⁄Manassas, VA, USA) and maintained in continuous culture using the Trager and Jensen (15) method at 5% hematocrit using type A+ human erythrocytes in RPMI 1640 medium (Sigma-Aldrich, St Louis, MO, USA) supplemented with 2 mM L-glutamine (Gibco, Eusébio, CE, Brazil), 25 mM HEPES (Sigma-Aldrich), 40 μg⁄mL gentamycin, 10% A+ human plasma, and 25 mM $NaHCO_3$. Cultures were maintained under a mixture of 5% O_2, 5% CO_2, and 90% N_2 and incubated at 37 °C. When cultures attained parasitemias of 4–5%, they were synchronized with 5% sorbitol (16).

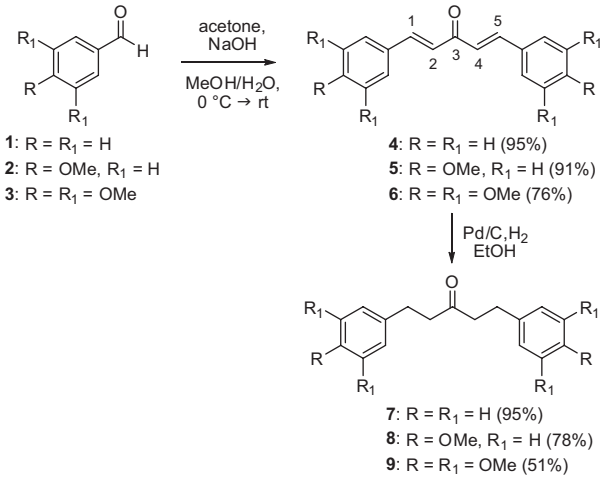
Test for *in vitro* inhibition of blood forms of *Plasmodium falciparum*

This assay was performed according to the method of Rieckmann *et al.* (17) with modifications described by Andrade-Neto *et al.* (18,19). Briefly, 5 mg⁄mL stock solutions of hydroperoxides **11–14** and **16** were prepared in DMSO. Stock solutions were serially diluted in culture medium (RPMI 1640) by a factor of 1:5 to obtain seven dilutions of each sample having final (sample well) concentrations of 100–6.4 × 10^{-3} μg⁄mL. Each diluted sample was tested in duplicate. Diluted samples were transferred to 96-well microtest plates containing parasitized red blood cell (RBC) suspension with 3% hematocrit and initial parasitemia of 1% of synchronized young trophozoites (ring form). Control wells contained 1% final concentration of DMSO. The final volume in each well was 200 μL. Reference antimalarial compounds (chloroquine and quinine) were tested in the concentrations recommended by WHO (20). The microplate was incubated for 48 h at 37 °C under a mixture of gases (5% O_2,

5% CO_2, and 90% N_2). After the incubation period, thin smears of the contents of each well were evaluated by optical microscopy (100× magnification). In this procedure, the number of parasites present in a total of approximately 2000 RBCs was determined. Parasitemia was expressed as a percentage of the number of parasitized RBCs found in the total number of RBCs counted:

$$\text{Parasitemia (\%)} = \frac{\text{No. of parasitized RBCs} \times 100}{\text{No. of RBCs total}}$$

The half-maximal inhibitory (IC_{50}) responses as compared to the drug-free controls were estimated by interpolation using Microcal Origin software. The test concentration values in units of log μg/mL were plotted against parasite viability (ratio of the average parasitemias of test wells to the average parasitemias of the control wells) for each concentration using the sigmoidal fitting analysis function to generate a smooth curve. The IC_{50} value is obtained from the graph and corresponds to a viability of 0.5.



Scheme 2: Preparation of ketones **4–9**.

Results

Preparation of dibenzalacetones and their hydrogenated 1,2-bisphenylmethyl acetone derivatives

α,β-Unsaturated ketones **4**, **5**, and **6** are structurally analogous to curcumin and were obtained from benzaldehyde (**1**), 4-methoxybenzaldehyde (**2**), and 3,4,5-trimethoxybenzaldehyde (**3**), respectively, in 76–95% yields (Scheme 2). Ketones **7**, **8**, and **9** were prepared from dibenzylacetones **4**, **5**, and **6**, respectively, by hydrogenation in 51–93% yields (Scheme 2).

Ketones **4**–**9**, cyclohexanone, and *tert*-butylcyclohexanone were used as substrates in the synthesis of dihydroperoxides and tetraoxanes. For this purpose, four different methods of direct preparation were tested: (i) 30% H_2O_2 with I_2 in MeCN;(21) (ii) 30% H_2O_2 with methyltrioxorhenium (MTO) in trifluoroethanol (TFE) or CH_2Cl_2; (22) (iii) 30% H_2O_2 with HCl in CH_2Cl_2 and MeCN; (23) (iv) 30% H_2O_2 with phosphomolybdic acid (PMA) in Et_2O (24).

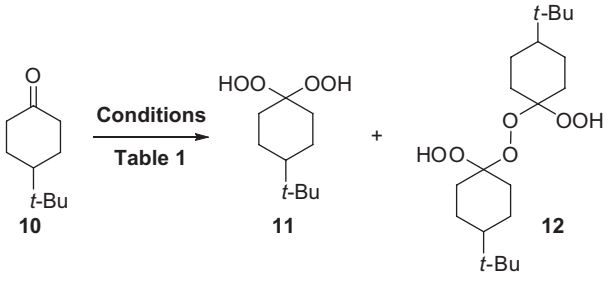
Synthesis of dihydroperoxides

Initial attempts to obtain dihydroperoxides were carried out starting with inexpensive and readily available *tert*-butylcyclohexanone applying the methods described above (Scheme 3). The results are summarized in Table 1.

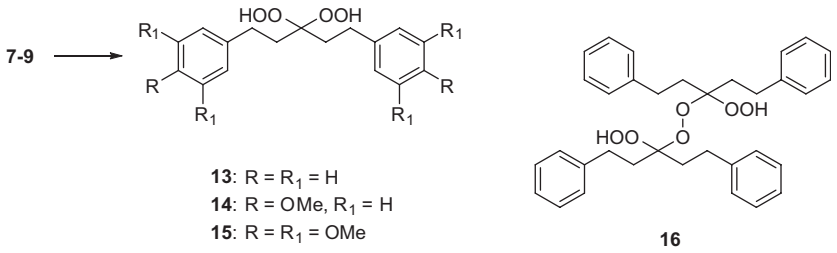
Preparation of dihydroperoxide was best performed with 30% H_2O_2 in Et_2O catalyzed by PMA (Entry 5) providing compound **11** in 89% yield. Using the MTO method, dihydroperoxide **11** was obtained along with dimeric dihydroperoxide **12**, which contains a peroxide function (Entries 2 and 3). Using these same conditions and starting

Table 1: Yields of dihydroperoxides **11** and **12** by treating 1 with 30% H_2O_2 under different conditions

		Yield (%)	
Entry	Method 30% H_2O_2	**11**	**12**
1	I_2, MeCN	61	–
2	MTO, TFE	30	18
3	MTO, CH_2Cl_2	18	41
4	HCl, CH_2Cl_2/MeCN	30	–
5	Et_2O, PMA	89	–



Scheme 3: Synthesis of dihydroperoxides **11** and **12** from 4-*tert*-butylcyclohexanone.



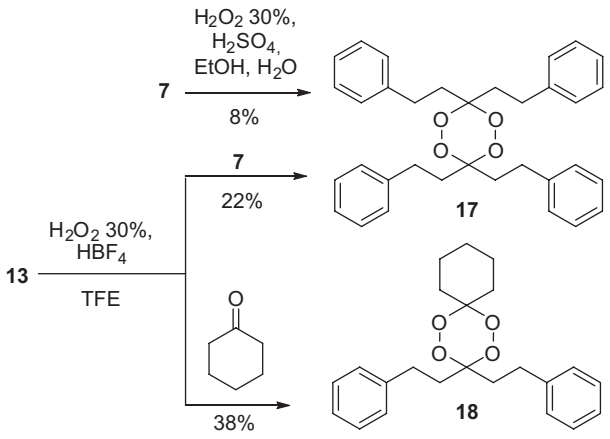
Scheme 4: Synthesis of dihydroperoxides **13–15** and dimer **16** from ketones **7–9**.

Table 2: Yields of dihydroperoxides **13–16** using H_2O_2 under different conditions

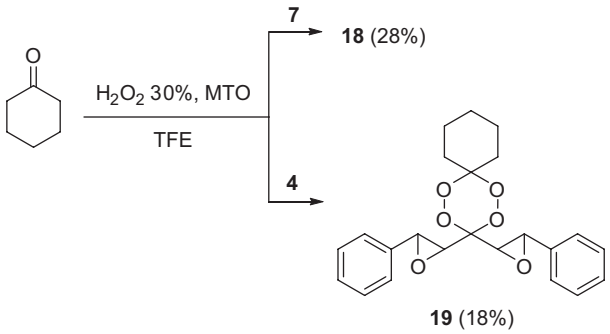
Entry	Method 30% H_2O_2	Substrate	Product	% Yield	Product	% Yield
1	I_2, MeCN	**7**	**13**	30	–	
2	MTO, TFE	**7**		15	**16**	11
3	MTO, CH_2Cl_2	**7**		15		23
4	HCl, CH_2Cl_2/MeCN	**7**		15	–	
5		**7**	**13**	84	–	
6	Et_2O, PMA	**8**	**14**	50	–	
7		**9**	**15**	31	–	

with ketones **7**–**9**, similar results were observed furnishing dihydroperoxides **13–15** and dimer **16** (Scheme 4, Table 2).

Thirty per cent H_2O_2/PMA in Et_2O was superior to other methods and provided **13** in good yield (84%) (Entry 5). Lower yields were obtained for the synthesis of analogs **14** and **15** (Entries 6 and 7). Other methods were less satisfactory providing yields of 15–30% (Entries 1–4). Dimer **16** was obtained as a side product during the synthesis of **13** using the MTO method. No dihydroperoxide derivatives were isolated when unsaturated ketones **4**–**6** were used as substrates in the methods described. Instead, oxidation or double bond cleavage products were observed.



Scheme 5: Synthesis of tetraoxanes **17** and **18**.



Scheme 6: Synthesis of tetraoxanes **18** and **19**.

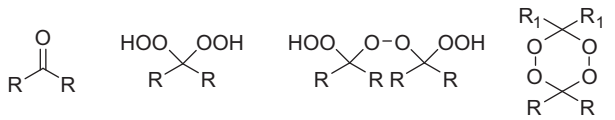
Synthesis of tetraoxanes

Three different methods were employed in the synthesis of tetraoxanes **17–19**. Symmetric tetraoxane **17** was prepared in 8% yield using 30% H_2O_2, H_2SO_4, EtOH, and H_2O (Scheme 5). A better yield of **17** (22%) was obtained by reacting dihydroperoxide **13** with ketone **7** in 30% H_2O_2 and HBF_4 in TFE. The reaction of **13** and cyclohexanone under the same conditions leads to non-symmetric tetraoxane **18** in 38% yield (Scheme 5).

Alternatively, the synthesis of non-symmetric tetraoxanes can be achieved directly from the corresponding ketones via a one-pot procedure described by Danièle-Bonnet and coworker (25). Treatment of a mixture of cyclohexanone and ketone **7** with 30% H_2O_2 and MTO/HBF_4 in TFE (Scheme 6) gave tetraoxane **18** in poor yield (28%). Using the same reaction conditions and a mixture of cyclohexanone and unsaturated ketone **4**, an unexpected tetraoxane diepoxide product **19** was isolated in 18% yield.

The biphenylidene acetone (**4**), three bi-benzylmethyl ketones **7**–**9**, three dihydroperoxides **11**, **13**, **14**, two peroxydihydroperoxides **12** and **16**, and two 1,2,4,5-tetroxanes **17** and **18** were initially screened at two concentrations for *in vitro* inhibition of the growth of blood stages of the human malaria parasite *P. falciparum* and hemolytic action. Dihydroperoxide **14** and **18** caused hemolysis of the human blood containing culture medium and were not further tested. Next, the nine remaining compounds were further tested at different concentrations to establish quantitative concentration–activity relationships and calculate the median inhibitory concentration (IC_{50}). Only compounds containing O–O bonds were found

Table 3: Inhibition of the *in vitro* growth of the K1 strain of *Plasmodium falciparum* by selected dihydroperoxide (B), dihydroperoxyperoxide (C), and 1,2,4,5-tetraoxane (D) compounds



Classes

Compound	Class	R	R_1	*P. falciparum*, IC_{50} (μM)	Result
4	A	*E*-PhCH=CH-	—	32	I
7	A	$PhCH_2CH_2$-	—	66	I
8	A	*p*-$CH_3OPhCH_2CH_2$-	—	42	I
9	A	3,4,5-$(CH_3O)_3PhCH_2CH_2$-	—	1.1×10^2	I
13	B	$PhCH_2CH_2$-	—	1.4	A
11	B	$(CH_3)_3CCH(CH_2CH_2\text{-})_2$	—	64	I
14	B	*p*-$CH_3OPhCH_2CH_2$-	—	Hemolysis	—
12	C	$(CH_3)_3CCH(CH_2CH_2\text{-})_2$	—	31	I
16	C	$PhCH_2CH_2$-	—	3.3	A
17	D	$PhCH_2CH_2$-	$PhCH_2CH_2$-	1.6	A
18	D	$Ph(CH_2)_2$-	-$(CH_2)_5$-	Hemolysis	—
CQ	—	—	—	0.25	VA
QN	—	—	—	0.012	VA

VA, very active (IC_{50} < 1.0 μM), A, active (1.0 ≤ IC_{50} ≤ 5 μM); I, inactive (IC_{50} > 10 μM); CQ, chloroquine; QN, quinine (controls).

to have significant antimalarial activities. Among the seven O-O-containing compounds which were tested, compounds **13**, **16**, and **17** (IC_{50} = 1.4–3.3 μM) were active (Table 3).

While specific mechanistic studies were not performed to establish the mode of antimalarial action of the compounds synthesized in this study, the preliminary antiplasmodial data presented in Table 3 allow for speculations as to the possible modes of action of the synthesized compounds. First, low antiplasmodial activity is associated with the starting bisbenzyl acetone (**7**), and its aryl ring substituted analogs **8** and **9** and no general cytotoxicity or membrane destabilization (hemolysis) was observed for bisbenzyl acetones **7**–**9**. On the other hand, high antiplasmodial activity was observed for *gem*-dihydroperoxide **13**, dihydroperoxy peroxide **16**, and 1,2,4,5-tetraoxane **17**, and hemolytic activity was observed for *gem*-dihydroperoxide **15** and tetraoxane **18**. These preliminary data may be an indication of a strong general interaction of this class of compound with the surface membranes of RBCs and perhaps those of free parasites (merozoites). Another possibility is that the highly active hydroperoxy and peroxide compounds are able to penetrate the erythrocyte and *P. falciparum* membranes and end up in the digestive vacuole of *P. falciparum* cells. This vacuole is the specific organelle wherein the infected RBC's hemoglobin (a protein) is digested by the parasite. An important by-product of hemoglobin digestion is heme. This substance, which is toxic to the parasite, is polymerized in the digestive vacuole to a stable hemozoin complex (26). There is evidence that common antimalarials such as chloroquine and artemisinin and highly antiplasmodial compounds having planar substructures such as ellipticine derivatives, xanthones act within the parasite digestive vacuole and kill the parasites by stabilizing soluble heme and preventing hemozoin formation (27). Furthermore, it is believed that ferric ion (toxic to parasites) metabolism is also affected in the digestive vacuole by the natural 1,2,4-trioxane (peroxide) artemisinin and its derivatives (28). The relatively flat or planar substructures and peroxy moieties in the synthesized compounds may thus be acting in the digestive vacuole by stabilizing heme/inhibiting hemozoin formation and also by affecting the metabolism of Fe^{3+}. Only through specific studies on mechanism and structure–activity relationships will the mechanism(s) of antiplasmodial action be forthcoming (29).

Conclusion

Synthesis of dihydroperoxides derived from hydrogenated dibenzalacetones was achieved using four different methods. The most efficient method was the treatment of ketone with hydrogen peroxide in ether catalyzed by phosphomolybdic acid, which provided the desired dihydroperoxides in 31–84% yields. Among the methods explored for the synthesis of tetraoxanes, the most efficient provided compounds **17** and **18** in 22% and 38% yield, respectively. Dihydroperoxide **13**, dihydroperoxy peroxide **16,** and 1,2,4,5-tetraoxane **17** exhibit significant antimalarial activity against chloroquine-resistant human malaria parasite *P. falciparum*. These compounds will be further evaluated for *in vivo* antimalarial and against other parasite species.

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