Synthetic Spirocyclic Endoperoxides: New Antimalarial Scaffolds

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Cyclohexanone 16\textsubscript{b} was also used as the starting material for the synthesis of five-membered spiro-derivative \textit{rac}-10 (Scheme 1 SI). Accordingly, 30 was condensed with 19 in the presence of LDA and the resulting alcohol intermediate was readily dehydrated to 31. Due to synthetic constrains, lactone 30 was reduced to the corresponding lactol which was protected as the methyl acetal intermediate 31. This latter compound underwent a hydroperoxysilylation reaction to 32, and after treatment with TMSOTf, the desired spiro-derivative \textit{rac}-10 was obtained.

\textbf{Scheme 1 SI}

\begin{equation}
\text{16b} \overset{a,b}{\rightarrow} \text{30} \overset{c,d}{\rightarrow} \text{31} \overset{f}{\rightarrow} \text{32} \overset{e}{\rightarrow} \text{rac-10}
\end{equation}

\textsuperscript{a}Reagents and conditions: (a) 19, LDA, 0 °C, 20 min, then 35, from -78 °C to 25 °C, 2 h; (b) SOCl\textsubscript{2}, pyridine, 25 °C, 2 h; (c) DIBAL, DCM, -78 °C, 1.5 h; (d) p-TsOH, MeOH, 25 °C, 2 h; (e) Co(thd)\textsubscript{2}, Et\textsubscript{3}SiH, O\textsubscript{2}, t-BuOOH (5 M in nonane), 1,2-DCE, 25 °C, 4 h; (f) TMSOTf, DCM, -78 °C, 5 min.
**Figure S1.** Docked poses of 1 (DHP) and ART (gray sticks; A, and B, respectively) in complex with heme (green balls and sticks), charged iron was coloured cyan and represented by CPK model. The potential ligand-metal coordination bonds are reported as green dotted lines. The picture was generated by Maestro.

**Experimental procedures**

General Procedures. Starting materials and solvents were purchased from commercial suppliers and used without further purification. Reaction progress was monitored by TLC using silica gel 60 F254 (0.040,0.063 mm) with detection by UV. Silica gel 60 (0.040,0.063 mm) was used for column chromatography. Yields refer to purified materials and are not optimized. $^1$H NMR and $^{13}$C NMR spectra were recorded on a Varian 300 MHz or a Bruker 400 MHz spectrometer using the residual signal of the deuterated solvent as internal standard. Splitting patterns are described as singlet (s), doublet (d), triplet (t), quartet (q), and broad (br); chemical shifts (δ) are given in ppm and coupling constants (J) in hertz (Hz). Mass spectra were recorded utilizing electrospray ionization (ESI). All moisture-sensitive reactions were performed under argon atmosphere using oven-dried glassware and anhydrous solvents. R* and S* indicate relative configurations.

**$^{(E/Z)}$-(3R,S)-3-(3-Adamantan-1-yl-2-methylallyl)-3-methyldihydrofuran-2(3H)-one (12a).** To a stirred solution of lithium bis(trimethylsilyl)amide (1.0 M in THF, 3.4 mL, 3.4 mmol) and THF (10 mL), cooled to -78 °C, a solution of 19 (0.31 g, 3.1 mmol) in THF (5.0 mL) was added dropwise. The reaction mixture was stirred at -78 °C for 30 min, afterward a solution of 18a (1.2 g, 3.7 mmol) in THF (5.0 mL) was added dropwise and the resulting reaction mixture was stirred at the same temperature for 10 min and then allowed to warm to
25 °C and stirred for further 2 h. A saturated solution of ammonium chloride was added to the reaction mixture and the aqueous layer was extracted with DCM. The combined organic extracts were dried over sodium sulfate, and the solvent was removed. The residue was purified by flash chromatography (1:10 ethyl acetate/n-hexane) to afford 12a as a white low melting solid and as a 3:2 mixture of geometric isomers (53% yield); 1H NMR (400 MHz, CDCl₃) δ 5.01 (s, 1H), 4.93 (s, 1H), 4.30 – 4.22 (m, 2H), 4.20 – 4.06 (m, 2H), 2.92 (d, J = 14.2 Hz, 1H), 2.37 – 2.22 (m, 3H), 2.12 (d, J = 13.5 Hz, 1H), 1.96 – 1.78 (m, 11H), 1.77 – 1.49 (m, 28H), 1.20 (s, 3H), 1.15 (s, 3H); MS (ESI) m/z 311 (M + Na)⁺; HRMS (ESI) calcd for (C₁₉H₂₈NaO₂)⁺: 311.1982; found: 311.1988.

(3R,S)-3-(2-Cyclohexylallyl)-3-methylidihydrofuran-2(3H)-one (12b). Starting from 22a and 19, the title compound was prepared following the procedure described for the synthesis of 12a and was obtained as colorless oil (24% yield); 1H NMR (300 MHz, CDCl₃) δ 4.86 (s, 1H), 4.75 (s, 1H), 4.24 – 4.09 (m, 2H), 2.46 (d, J = 14.2 Hz, 1H), 2.37 – 2.25 (m, 1H), 2.15 (d, J = 11.3 Hz, 1H), 2.06 – 1.83 (m, 1H), 1.86 – 1.59 (m, 6H), 1.21 (s, 3H), 1.18 – 0.93 (m, 5H); MS (ESI) m/z 245 (M + Na)⁺; MS (ESI) m/z 245 (M + Na)⁺; HRMS (ESI) calcd for (C₁₄H₂₂NaO₂)⁺: 245.1512; found: 245.1512.

(3R,S)-3-(2-Cyclopentylallyl)-3-methylidihydrofuran-2(3H)-one (12c). Starting from 22c and 19, the title compound was prepared following the procedure described for the synthesis of 12a and was obtained as colorless oil (60% yield). 1H NMR (400 MHz, CDCl₃) δ 4.90 (s, 1H), 4.73 (s, 1H), 4.26-4.08 (m, 2H), 2.50 – 2.40 (m, 2H), 2.23 (m, 2H), 1.97 – 1.91 (m, 1H), 1.87 – 1.77 (m, 2H), 1.69 – 1.59 (m, 2H), 1.59 – 1.48 (m, 2H), 1.39 – 1.30 (m, 2H), 1.22 (s, 3H); MS (ESI) m/z 231 (M + Na)⁺; HRMS (ESI) calcd for (C₁₃H₂₀NaO₂)⁺: 231.1356; found: 231.1359.

(3R,S)-3-(Cyclohexen-1-ylmethyl)-3-methylidihydrofuran-2(3H)-one (12d). Starting from 19 and 18b, the title compound was prepared following the procedure described for the synthesis of 12a and was obtained as colorless oil (73% yield); 1H NMR (300 MHz, CDCl₃) δ 5.49 (s, 1H), 4.15 (t, J = 7.1 Hz, 2H), 2.35 – 2.19 (m, 2H), 2.08 (d, J = 13.7 Hz, 1H), 2.00 – 1.77 (m, 5H), 1.63 – 1.36 (m, 4H), 1.16 (s, 3H); MS (ESI) m/z 217 (M + Na)⁺; HRMS (ESI) calcd for (C₁₂H₁₈NaO₂)⁺: 217.1199; found: 217.1192.

(3R,S)-3-(Cyclopentylidenemethyl)-3-methylidihydrofuran-2(3H)-one (13a). Thionyl chloride (1.6 mL, 21.5 mmol) was added to a solution of 27a (1.20 g, 6.06 mmol) in pyridine, cooled to 0 °C. The resulting mixture was stirred at 25 °C for 2 h, afterward a 1 N solution of hydrochloric acid was added. The aqueous phase was extracted with DCM. The organic extracts were dried over sodium sulfate, and the solvent was evaporated. The crude product
was purified by flash chromatography (1:10 ethyl acetate/petroleum ether 40-60) to afford 13a (100% yield) as colorless oil. $^1$H NMR (400 MHz, CDCl$_3$) $\delta$ 5.29 (s, 1H), 4.19 – 4.06 (m 2H), 2.32 – 2.00 (m, 6H), 1.64 – 1.49 (m, 2H), 1.49 – 1.35 (m, 2H), 1.22 (d, $J$ = 10.0 Hz, 3H); MS (ESI) $m/z$ 203 (M + Na)$^+$; HRMS (ESI) calcd for (C$_{11}$H$_{16}$NaO$_2$)$^+$: 203.1043; found: 203.1044.

(3$R$,S)-3-(2-Adamantylidenemethyl)-3-methylidihydrofuran-2(3$H$)-one (13b). Starting from 27b, the title compound was prepared following the procedure described for the synthesis of 13a and was obtained as white solid (67% yield); $^1$H NMR (400 MHz, CDCl$_3$) $\delta$ 5.19 (s, 1H), 4.28 – 4.10 (m, 2H), 2.69 (m br, 1H), 2.32 – 2.13 (m, 3H), 1.90 – 1.66 (m, 12H), 1.30 (s, 3H); MS (ESI) $m/z$ 269 (M + Na)$^+$; HRMS (ESI) calcd for (C$_{16}$H$_{22}$NaO$_2$)$^+$: 269.1512; found: 269.1509.

(3$R$,S)-3-(2-Cyclohexylideneneethyl)-3-methylidihydrofuran-2(3$H$)-one (13c). Starting from 19 and 18c, the title compound was prepared following the procedure described for the synthesis of 12a and was obtained as colorless oil (23% yield); $^1$H NMR (300 MHz, CDCl$_3$) $\delta$ 5.04 (t, $J$ = 7.8 Hz, 1H), 4.27 – 4.18 (m, 2H), 2.32 – 1.98 (m, 7H), 1.99 – 1.84 (m, 2H), 1.63 – 1.40 (m, 5H), 1.23 (s, 3H); MS (ESI) $m/z$ 231 (M + Na)$^+$; HRMS (ESI) calcd for (C$_{13}$H$_{20}$NaO$_2$)$^+$: 231.1356; found: 231.1356.

(3$R$,S,2$R$,S)-3-(3-(Adamantan-1-yl)-2-methyl-2-(triethylsilylperoxy)propyl)-3-methylidihydrofuran-2(3$H$)-one (14a). To a solution of 12a (0.78 g, 2.7 mmol) and Co(thd)$_2$ (0.34 g, 0.81 mmol) in 1,2-DCE (20 mL), stirred under an oxygen atmosphere, triethylsilane (5.4 mmol, 0.86 mL) together with a drop of tert-butylhydroperoxide (5.0 M in nonane, catalytic amount) were added. The resulting green mixture was stirred at 25 °C for 4 h, until consumption of the starting material. Thereafter the solvent was evaporated and the resulting residue was purified by flash chromatography (1:20 ethyl acetate/n-hexane) to afford 14a (80% yield) as a pale green oil and as a mixture of diastereoisomers; $^1$H NMR (300 MHz, CDCl$_3$) $\delta$ 4.43 – 4.11 (m, 4H), 3.00 – 2.76 (m, 4H), 2.35 – 1.82 (m, 12H), 1.82 – 1.50 (m, 20H), 1.49 – 1.12 (m, 18H), 1.13 – 0.80 (m, 18H), 0.81 – 0.55 (m, 12H); MS (ESI) $m/z$ 459 (M + Na)$^+$; HRMS (ESI) calcd for (C$_{25}$H$_{44}$NaO$_4$Si)$^+$: 459.2901; found: 459.2903.

(3$R$,S,2$R$,S)-3-(2-Cyclohexyl-2-(triethylsilylperoxy)propyl)-3-methylidihydrofuran-2(3$H$)-one (14b). Starting from 12b, the title compound 14b was prepared as described for the synthesis of 14a and was obtained as colorless oil; $^1$H NMR (300 MHz, CDCl$_3$) $\delta$ 4.36 – 4.14 (m, 4H), 2.89 – 2.65 (m, 2H), 2.05 – 1.55 (m, 20H), 1.35 – 1.07 (m, 20H), 1.02 – 0.90 (m, 18H), 0.77 – 0.50 (m, 12H); MS (ESI) $m/z$ 393 [M + Na]$^+$, 371 [M + H]$^+$; HRMS (ESI) calcd for (C$_{20}$H$_{38}$NaO$_4$Si)$^+$: 393.2432; found: 393.2437.
\((3R,3'S,2'R,S)-3\text{-}(2\text{-Cyclopentyl}-2\text{-}(\text{triethylsilylperoxy})\text{propyl})\text{-}3\text{-methylidihydrofuran-2}(3H)\text{-one (14c)}\). Starting from 12c, the title compound 14c was prepared as described for the synthesis of 14a and was obtained as colorless oil; \(^1\)H NMR (400 MHz, CDCl\(_3\)) \(\delta\) 4.37 - 4.25 (m, 2H), 4.25 - 4.14 (m, 2H), 2.90 - 2.69 (m, 2H), 2.42 - 1.81 (m, 10H), 1.74 - 1.42 (m, 14H), 1.39 - 1.12 (m, 12H), 0.94 (t, \(J = 7.9\) Hz, 18H), 0.64 (t, \(J = 7.9\) Hz, 12H).

MS (ESI) \(m/z\) 379 [M + Na]\(^+\); HRMS (ESI) calcd for \((C_{19}H_{36}NaO_4)\): 379.2275; found: 379.2273.

\((3R,S)-3\text{-Methyl}-3\text{-}((\text{triethylsilylperoxy})\text{cyclohexyl})\text{methyl})\text{dihydrofuran-2}(3H)\text{-one (14d)}\). Starting from 12d, the title compound 14d was prepared as described for the synthesis of 14a and was obtained as colorless oil; \(^1\)H NMR (300 MHz, CDCl\(_3\)) \(\delta\) 4.20 (m, 2H), 3.67 (s, 1H), 2.84 (s, 1H), 2.30 - 2.01 (m, 1H), 1.97 - 1.63 (m, 5H), 1.59 - 1.01 (m, 9H), 0.89 (s, 9H), 0.75 - 0.38 (m, 6H); MS (ESI) \(m/z\) 365 (M + Na); HRMS (ESI) calcd for \((C_{18}H_{34}NaO_4)\): 365.2119; found: 365.2113.

\((3R,S)-3\text{-Methyl}-3\text{-}((1\text{-triethylsilylperoxycyclopentyl})\text{methyl})\text{dihydrofuran-2}(3H)\text{-one (15a)}\). Starting from 13a, the title compound was prepared following the procedure described for the synthesis of 14a and was obtained as pale green oil (54% yield); \(^1\)H NMR (400 MHz, CDCl\(_3\)) \(\delta\) 4.26 (td, \(J = 8.6, 5.1\) Hz, 1H), 4.16 (dd, \(J = 16.1, 7.8\) Hz, 1H), 2.76 (dt, \(J = 13.0, 7.9\) Hz, 1H), 2.17 (d, \(J = 15.1\) Hz, 1H), 2.07 - 1.84 (m, 3H), 1.84 - 1.69 (m, 2H), 1.69 - 1.33 (m, 5H), 1.21 (s, 3H), 0.90 (t, \(J = 7.9\) Hz, 9H), 0.59 (q, \(J = 7.9\) Hz, 6H); MS (ESI) \(m/z\) 351 (M + Na); HRMS (ESI) calcd for \((C_{17}H_{32}NaO_4)\): 351.1962; found: 351.1966.

\((3R,S)-3\text{-Methyl}-3\text{-}((2\text{-triethylsilylperoxy}-2\text{-adamantyl})\text{methyl})\text{dihydrofuran-2}(3H)\text{-one (15b)}\). Starting from 13b, the title compound was prepared following the procedure described for the synthesis of 14a and was obtained as white solid (25% yield); \(^1\)H NMR (400 MHz, CDCl\(_3\)) \(\delta\) 3.73 - 3.60 (m, 2H), 2.24 - 2.09 (m, 4H), 1.89 (d, \(J = 9.1\) Hz, 2H), 1.84 - 1.71 (m, 4H), 1.70 - 1.58 (m, 3H), 1.52 - 1.40 (m, 2H), 1.29 (s, 3H), 1.10 (s, 3H), 0.88 (t, \(J = 7.9\) Hz, 9H), 0.52 (q, \(J = 7.9\) Hz, 6H); MS (ESI) \(m/z\) 417 (M + Na); HRMS (ESI) calcd for \((C_{22}H_{38}NaO_4)\): 417.2432; found: 417.2433.

\((3R,S)-3\text{-Methyl}-3\text{-}((3\text{-triethylsilylperoxy}-3\text{-cyclohexyl})\text{ethyl})\text{dihydrofuran-2}(3H)\text{-one (15c)}\). Starting from 13c, the title compound 15c was prepared as described for the synthesis of 14a and was obtained as colorless oil; \(^1\)H NMR (300 MHz, CDCl\(_3\)) \(\delta\) 4.32 - 4.14 (m, 2H), 2.30 - 2.18 (m, 1H), 2.08 - 1.92 (m, 1H), 1.81 - 1.25 (m, 14H), 1.23 (s, 3H), 1.00 - 0.88 (m, 9H), 0.71
promoted cyclization, the title compound *rac-3* was prepared as described for the synthesis of *rac-3* and was obtained as colorless oil and as a mixture of diastereoisomers (73% yield over two steps); 1H NMR (300 MHz, CDCl₃) δ 5.05 (s, 1H), 4.98 (s, 1H), 4.30 – 3.9 (m, 2H), 2.26 – 2.12 (m, 2H), 2.01 (d, J = 14.2 Hz, 1H), 1.94 – 1.52 (m, 2H), 1.33 – 1.17 (m, 8H), 1.07 (s, 3H), 1.04 (s, 3H); 13C NMR (75 MHz, CDCl₃) δ 110.5, 110.3, 82.0, 81.8, 68.6, 68.5, 55.9, 55.0, 44.9, 44.8, 44.4, 44.1, 40.6, 40.4, 37.2, 37.1, 36.9, 36.3, 34.1, 33.7, 29.1 (2C), 27.6, 27.5 (2C), 27.0; MS (ESI) m/z 329 (M + Na)⁺, 635 (2M + Na)⁺; HRMS (ESI) calcd for (C₁₉H₃₀NaO₃)⁺: 329.2087; found: 329.2087. El. Anal. calcd for C₁₉H₃₀O₃: C, 74.47; H, 9.87; found: C, 74.68; H, 9.80.

**\(3R^*,4aS^*,7aR^*)\)-3-Cyclohexyl-3,4a-dimethyltetrahydrofuro[2,3-c][1,2]dioxane and \(3S^*,4aS^*,7aR^*)\)-3-Cyclohexyl-3,4a-dimethyltetrahydrofuro[2,3-c][1,2]dioxane (rac-4).**

Starting from 14b, through DIBAL-promoted reduction of the lactone to lactol, and TMSOTf-promoted cyclization, the title compound *rac-4* was prepared as described for the synthesis of *rac-3* and was obtained as colorless oil and as a mixture of diastereoisomers (73% yield over two steps); 1H NMR (300 MHz, CDCl₃) δ 5.05 (s, 1H), 4.98 (s, 1H), 4.30 – 4.11 (m, 2H), 4.03 – 3.90 (m, 2H), 2.43 – 2.28 (m, 2H), 2.26 – 2.12 (m, 2H), 2.01 (d, J = 14.2 Hz, 1H), 1.94 – 1.52 (m, 1H), 1.33 – 1.17 (m, 8H), 1.07 (s, 3H), 1.04 (s, 3H); 13C NMR (75 MHz, CDCl₃) δ 110.5, 110.3, 82.0, 81.8, 68.6, 68.5, 55.9, 55.0, 44.9, 44.8, 44.4, 44.1, 40.6, 40.4, 37.2, 37.1, 36.9, 36.3, 34.1, 33.7, 29.1 (2C), 27.6, 27.5 (2C), 27.0; MS (ESI) m/z 329 (M + Na)⁺, 635 (2M + Na)⁺; HRMS (ESI) calcd for (C₁₉H₃₀NaO₃)⁺: 329.2087; found: 329.2087. El. Anal. calcd for C₁₉H₃₀O₃: C, 74.47; H, 9.87; found: C, 74.68; H, 9.80.
18H), 1.40 (d, J = 14.3 Hz, 1H), 1.16 (s, 3H), 1.13 (s, 3H), 1.11 (s, 6H), 1.06 – 0.85 (m, 6H); 13C NMR (75 MHz, CDCl₃) δ 110.4, 109.9, 82.1, 81.9, 68.7, 68.2, 47.3, 46.5, 41.7, 40.7, 40.6, 40.0, 38.2, 36.4, 27.9, 27.6, 27.2, 27.0, 26.8, 26.6, 26.5, 26.4, 20.7, 20.6; MS (ESI) m/z 263 (M + Na)⁺; HRMS (ESI) calcd for (C₁₄H₂₄NaO₃)⁺: 263.1618; found: 263.1621. El. Anal. calcd for C₁₄H₂₄O₃: C, 69.96; H, 10.07; found: C, 70.14; H, 10.46.

(3R*,4aS*,7aR*)-3-Cyclopentyl-3,4a-dimethyltetrahydrofuro[2,3-c][1,2]dioxane and (3S*,4aS*,7aR*)-3-Cyclopentyl-3,4a-dimethyltetrahydrofuro[2,3-c][1,2]dioxane (rac-5). Starting from 14c, through DIBAL-promoted reduction of the lactone to lactol, and TMSOTf-promoted cyclization, the title compound rac-5 was prepared as described for the synthesis of rac-3 and was obtained as colorless oil and as a mixture of diastereoisomers (41% yield over two steps); 1H NMR (300 MHz, CDCl₃) δ 5.05 (s, 1H), 5.01 (s, 1H), 4.22-4.10 (m, 2H), 4.01-3.95 (m, 2H), 2.40 – 2.20 (m, 4H), 2.00 – 1.80 (m, 3H), 1.70 – 1.55 (m, 16H), 1.20 (s, 6H), 1.15 (s, 3H), 1.12 (s, 3H); 13C NMR (75 MHz, CDCl₃) δ 109.9, 82.2, 68.6, 68.2, 49.0, 47.8, 41.7, 41.5, 40.6, 39.7, 38.3, 36.4, 28.1, 27.7, 27.4, 27.1, 26.7, 26.3, 26.22, 26.1, 25.8, 21.3, 20.9; MS (ESI) m/z 227 (M + H)⁺, 249 (M + Na)⁺; HRMS (ESI) calcd for (C₁₃H₂₂O₃)⁺: 227.1642; found: 227.1639. El. Anal. calcd for C₁₃H₂₂O₃: C, 68.99; H, 9.80; found: C, 68.79; H, 9.86.

(4a'S*,7a'R*)-4a'-Methyltetrahydrospiro[cyclohexane-1,3-furo[2,3-c][1,2]dioxane (rac-6). Starting from 14c, through DIBAL-promoted reduction of the lactone to lactol, and TMSOTf-promoted cyclization, the title compound rac-6 was prepared as described for the synthesis of rac-3 and was obtained as colorless oil (34% yield over two steps); 1H NMR (400 MHz, CDCl₃) δ 4.95 (s, 1H), 4.24 – 4.11 (m, 1H), 3.91 (q, J = 7.9 Hz, 1H), 2.31 – 2.16 (m, 1H), 1.90 – 1.72 (m, 3H), 1.68 – 1.49 (m, 3H), 1.48 – 1.31 (m, 6H), 1.29 – 1.16 (m, 1H), 1.07 (s, 3H); 13C NMR (75 MHz, CDCl₃) δ 110.3, 79.0, 68.4, 42.0, 40.5, 37.2, 37.1, 36.9, 27.4, 25.7, 22.4, 22.2; MS (ESI) m/z 235 (M + Na)⁺, 447 (2M + Na)⁺; HRMS (ESI) calcd for (C₁₂H₂₀NaO₃)⁺: 235.1305; found: 235.1299. El. Anal. calcd for C₁₂H₂₀O₃: C, 67.89; H, 9.50; found: C, 67.51; H, 9.50.

(4a'S*,7a'R*)-4a'-Methyltetrahydro-4H-spiro[cyclopentane-1,3-furo[2,3-c][1,2]dioxane (rac-7). Starting from 15a, through DIBAL-promoted reduction of the lactone to lactol, and TMSOTf-promoted cyclization, the title compound was prepared following the procedure described for the synthesis of rac-3 and was obtained as colorless oil (58% yield over two steps); 1H NMR (300 MHz, CDCl₃) δ 5.02 (s, 1H), 4.26 – 4.15 (m, 1H), 4.00 (q, J = 8.0 Hz, 1H), 2.30 – 2.13 (m, 1H), 2.09 – 2.00 (m, 2H), 1.99 – 1.79 (m, 3H), 1.70 – 1.49 (m, 5H), 1.48 – 1.36 (m, 1H), 1.11 (s, 3H); 13C NMR (75 MHz, CDCl₃) δ 110.0, 89.2, 68.7, 42.1, 40.5, 39.1, 38.2, 35.5,
δ 5.21 (s, 1H), 4.27
acetate/was evaporated. The crude residue was purified by flash chromatography (1:30 ethyl acetate/n-hexane) to afford ester 17a as colorless oil (51% yield); 1H NMR (300 MHz, CDCl3) δ 5.11 (s, 1H), 4.27 – 4.09 (m, 2H), 2.03 – 1.81 (m, 6H), 1.79 – 1.63 (m, 10H), 1.39 – 1.21 (m,
consistent with those described in the literature.

The combined organic extracts were dried over sodium sulfate and the solvent was removed. The white precipitate was filtered off, and the aqueous phase was extracted with DCM. The white precipitate was filtered off, and the aqueous phase was extracted with DCM. The combined organic extracts were dried over sodium sulfate and the solvent was removed.

2-Cyclohexylideneacetic acid ethyl ester (17b). Starting from 16b and triethyl phosphonoacetate, the title compound was prepared following the procedure described for the synthesis of 17a and was obtained as white low melting solid (71% yield). Physical and spectroscopic data are consistent with those reported in the literature.\(^1\)

\((E/Z)-2\)-Methyl-3-(1-adamantyl)-1-iodoprop-2-ene (18a). To a solution of 17a (5.7 g, 24.4 mmol) in dry DCM (50 mL), diisobutyaluminium hydride (48.9 mL, 48.9 mmol) was added at -78 °C and the resulting mixture was stirred at -78 °C for 1.5 h. Afterward, water was added to the reaction mixture and the mixture was allowed to warm to 25 °C and stirred for 10-15 min. The white precipitate was filtered off, and the aqueous phase was extracted with DCM. The combined organic extracts were dried over sodium sulfate and the solvent was removed. The crude residue was purified by flash chromatography (1:20 ethyl acetate/n-hexane) to give (E/Z)-3-(adamantan-1-yl)-2-methylprop-2-en-1-ol (76% yield) as a white low melting solid; \(^1\)H NMR (400 MHz, CDCl\(_3\)) \(\delta\) 5.07 (s, 1H), 5.01 (s, 1H), 4.15 (s, 2H), 3.82 (s, 2H), 1.87 (s, 6H), 1.82 – 1.52 (m, 32H); MS (ESI) \(m/z\) 229 (M + Na); To a mixture of triphenylphosphine (3.4 g, 13.0 mmol) and imidazole (0.97 g, 14.4 mmol) in 1:3 acetonitrile/diethyl ether (22 mL), iodine was added (3.1 g, 13.0 mmol) in two portions at 0 °C and the resulting suspension was stirred at 0 °C for 30 min, when a yellow precipitate was formed. A solution of above alcohol (0.88 g, 4.3 mmol) in 1:3 acetonitrile/diethyl ether (4.0 mL) was subsequently added and the resulting mixture was stirred at 0 °C for 30 min. After this time, a 1:6 mixture of diethyl ether/n-hexane (30 mL) was added, and the resulting precipitate was filtered off. The filtrate was evaporated under vacuum, and the crude residue was filtered through a pad of silica gel which was washed with 1% ethyl acetate in n-hexane. The filtrate was evaporated and the residue 18a was immediately used for the next reaction.

(2-Iodoethylidene)cyclohexane (18b). To a solution of 17b (1.10 g, 6.5 mmol) in dry DCM (10 mL), cooled to -78 °C, was added dropwise a 1.0 M solution of DIBAL (13.0 mL, 13 mmol) and the resulting reaction mixture was stirred at -78 °C for 1.5 h. Subsequently, water was added and the resulting mixture was allowed to warm to 25 °C and stirred for further 10-15 min. The white precipitate was filtered off, and the aqueous phase was extracted with DCM. The combined organic extracts were dried over sodium sulfate and the solvent was removed to afford the crude alcohol as pale yellow oil (0.56 mg, 69%), whose spectroscopic data are consistent with those described in the literature.\(^2\) The above alcohol (0.30 g, 2.4 mmol) was...
dissolved in dioxane (10 mL) and boron trifluoride diethyl etherate (0.34 g, 2.4 mmol) and potassium iodide (0.39 g, 2.4 mmol) were added to the resulting solution. The reaction mixture was stirred at 25 °C for 3 h and subsequently poured into ice/water. The aqueous phase was extracted with diethyl ether and the organic phase was dried over sodium sulfate. The solvent was removed under reduced pressure and the crude compound (100%) was directly used in the next step without further purification. MS (ESI) m/z 259 (M + Na)+.

1-(Iodomethyl)cyclohexene (18c). Compound 17c was reduced as described for the synthesis of 17a to afford cyclohexenylmethanol (2.3 g, 73%) as a clear viscous liquid. Physical and spectroscopic data are consistent with those reported in the literature. The above alcohol was iodinated as described for the synthesis of 17a and it was obtained as brownish oil (44% yield), which was immediately used for the next reaction.

3-Chloro-2-cyclohexylpropene (21a). To a vigorously stirred mixture of 20a (0.72 g, 5.8 mmol) and CeCl₃•7H₂O (4.31 g, 11.6 mmol) in a 1:1 mixture of DCM/H₂O an aqueous solution of NaOCl (10-13%, 0.72 mL, 11.6 mmol) was added dropwise. The reaction mixture was stirred for 14 h, afterward it was treated with a saturated solution of Na₂S₂O₃ and the aqueous phase was extracted with DCM. The organic phase was dried over sodium sulfate, the solvent was removed in vacuo and the crude residue was purified by flash chromatography (n-hexane) to afford 21a (47% yield) as colorless oil; ¹H NMR (300 MHz, CDCl₃) δ 4.96 (s, 1H), 4.66 (s, 1H), 4.08 (s, 2H), 2.18-2.03 (m, 1H), 1.92 – 1.52 (m, 5H), 1.45 – 1.06 (m, 5H); MS (ESI) m/z 181 (M + Na)+; HRMS (ESI) calcd for (C₉H₁₅ClNa)⁺: 181.0754; found: 181.0750.

3-Chloro-2-(1-adamantyl)-propene (21b). Starting from 20b, the title compound was prepared following the procedure described for the synthesis of 21a and was obtained as colorless oil (96% yield); ¹H NMR (400 MHz, CDCl₃) δ 5.16 (s, 1H), 5.03 (s, 1H), 4.04 (s, 1H), 1.99 – 1.82 (m, 2H), 1.77 – 1.45 (m, 14H); MS (ESI) m/z 233 (M + Na)+; HRMS (ESI) calcd for (C₁₃H₁₉ClNa)+: 233.1067; found: 233.1070.

3-Iodo-2-cyclohexylpropene (22a). To a solution of 21a (0.43 mg, 2.7 mmol) in acetone (6.0 mL), NaI (0.41 g, 2.7 mmol) was added and the reaction mixture was stirred at 25 °C for 18 h. The solid residue was filtered off and washed with acetone. The organic solvent was removed and the residue was dissolved in DCM and washed with 10% Na₂S₂O₃ and water. The organic phase was dried over sodium sulfate and the solvent was removed. The crude compound 22a was used in the next step without further purification; MS (ESI) m/z 273 (M + Na)+.
3-Iodo-2-(1-adamantyl)-propene (22b). Starting from 21b, the title compound was prepared following the procedure described for the synthesis of 22a and was obtained as pale yellow oil (57% yield); MS (ESI) m/z 325 (M + Na)+.

3-Iodo-2-cyclopentylpropene (22c). To a stirred solution of 25 (200 mg, 0.17 mmol) in dry diethyl ether (5.0 mL), triethylamine (0.7 mL, 0.51 mmol) and methanesulfonyl chloride (0.19 mL, 2.4 mmol) were added at 0 °C. After 12 h, the reaction mixture was warmed to 25 °C, quenched by addition of 1 N HCl and water, and the aqueous phase was extracted with diethyl ether. The organic extracts were dried over sodium sulfate, and the solvent was evaporated. The crude product was purified by flash chromatography (1:10 ethyl acetate/petroleum ether 40-60) to afford 2-cyclopentylallyl methanesulfonate as colorless oil (0.2 g, 100% yield). 1H NMR (400 MHz, CDCl3) δ 5.09 (s, 1H), 5.03 (s, 1H), 4.62 (s, 2H), 2.95 (s, 3H), 2.42 (m, 1H), 1.87 – 1.77 (m, 2H), 1.69 – 1.59 (m, 2H), 1.59 – 1.48 (m, 2H), 1.39 – 1.30 (m, 2H); 13C NMR (75 MHz, CDCl3) δ 145.7, 113.5, 72.6, 43.1, 37.9, 31.5, 25.0. To a solution of the above compound (0.20, 1.1 mmol) in diethyl ether (5.0 mL), lithium iodide (0.28 g, 0.34 mmol) was added and the reaction mixture was sonicated at 25 °C for 3 h. Thereafter, the organic phase was washed with water, dried over sodium sulfate and concentrated in vacuo. The title compound was obtained as yellow oil and used in the next step without further purification (0.2 g, 100% yield).

2-Cyclopentyl-2-propenol (25). To a stirred solution of cyclopentylmagnesium bromide 23 (26.8 mmol) in dry THF (5.0 mL) cooled to -10 °C, a solution of propargyl alcohol 24 in dry THF (2.0 mL) was added dropwise, followed by the addition of copper(I) iodide (1.8 mmol). The reaction mixture was stirred at 25 °C for 24 h and at 80 °C for 5 h. Afterward the reaction was cooled to 0 °C and it was quenched by addition of an ice-NH4Cl saturated solution. The volatiles were removed in vacuo and the residue was filtered through celite. The aqueous phase was extracted with diethyl ether. The organic solvent was dried over sodium sulfate and concentrated in vacuo. The crude residue was purified by flash chromatography (1:5 ethyl acetate/petroleum ether 40-60). The title compound was obtained as colorless oil (50% yield); physical and spectroscopic data are consistent with those reported in the literature.4

(3R,S)-3-(Cyclopentyl(hydroxy)methyl)-3-methyldihydrofuran-2(3H)-one (27a). n-Butyllithium (7.65 mL, 1.6 M in THF, 12.2 mmol) was added dropwise to a solution of diisopropylamine (1.74 mL, 12.2 mmol) in THF (10 mL) at 0 °C. After 30 min, the reaction mixture was cooled down to -78 °C and a solution of 19 (1.02 g, 10.2 mmol) in THF (5.0 mL) was added dropwise. After 20 min, the reaction mixture was warmed to -40 °C and a solution
of 26a (1.00 g, 10.2 mmol) in THF (5.0 mL) was added. After 1 h the reaction mixture was warmed to 25 °C and stirred until completion. The reaction was quenched by addition of a saturated solution of ammonium chloride, the volatiles were removed under reduced pressure and the aqueous phase was extracted with DCM. The organic extracts were dried over sodium sulfate, and the solvent was evaporated. The crude product was purified by flash chromatography (1:5 ethyl acetate/petroleum ether 40-60) to afford 27a (73%) as a pale yellow oil and as a mixture of diastereoisomers; \(^1\)H NMR (400 MHz, CDCl\(_3\)) \(\delta\) 4.30 – 4.21 (m, 2H), 4.15 (m, 2H), 3.82 (s, 2H), 2.60 (m, 2H), 2.20 (d, \(J = 4.8\) Hz, 2H), 2.05 – 1.90 (m, 2H), 1.82 – 1.34 (m, 18H), 1.15 (s, 6H); MS (ESI) \(m/z\) 221 (M + Na); HRMS (ESI) calcd for (C\(_{11}\)H\(_{18}\)NaO\(_3\))\(^+\): 221.1148; found: 221.1153.

(3R,S)-3-((2-Adamantyl)(hydroxy)methyl)-3-methylidihydrofuran-2(3H)-one (27b). Starting from 19 and 26b, the title compound was prepared following the procedure described for the synthesis of 27a and was obtained as white solid (25% yield); \(^1\)H NMR (300 MHz, CDCl\(_3\)) \(\delta\) 4.37 – 4.10 (m, 4H), 4.00 (t, \(J = 9.4\) Hz, 2H), 2.63 – 2.49 (m, 4H), 2.48 – 2.07 (m, 6H), 1.98 – 1.64 (m, 22H), 1.63 – 1.46 (m, 4H), 1.26 (s, 3H), 1.24 (s, 3H); MS (ESI) \(m/z\) 287 (M + Na); 551 (2M + Na); HRMS (ESI) calcd for (C\(_{16}\)H\(_{26}\)NaO\(_3\))\(^+\): 287.1618; found: 287.1618.

(2R,S)-2-(1’-Triethylsilylperoxycyclohexyl)-1-cyclohexanol (29). To a stirred suspension of sodium borohydride (0.5 g, 13.5 mmol) in ethanol (10 mL), a solution of commercially available ketone 28 (2.0 g, 11.2 mmol) in ethanol (10 mL) was added dropwise at 0 °C and the resulting reaction mixture was stirred at 25 °C until the completion of reaction. Subsequently, water was added and the volatiles were removed under reduced pressure. The aqueous phase was extracted with DCM, the organic extracts were dried over sodium sulfate and the solvent was removed. The crude residue was purified by flash chromatography (1:5 ethyl acetate/petroleum ether 40-60) to afford 2-(cyclohexen-1-yl)-1-cyclohexanol (1.3 g, 64%) as white solid; \(^1\)H NMR (400 MHz, CDCl\(_3\)) \(\delta\) 5.58 (s, 2H), 3.40 (tt, \(J = 9.7, 4.2\) Hz, 2H), 2.13 – 1.98 (m, 6H), 1.91 (d, \(J = 3.9\) Hz, 6H), 1.84 – 1.53 (m, 16H), 1.38 – 1.12 (m, 8H). Starting from the above compound, the title compound was prepared following the procedure described for the synthesis of 14a and was obtained as pale green oil and as a mixture of diastereoisomers (44% yield); \(^1\)H NMR (300 MHz, CDCl\(_3\)) \(\delta\) 4.36 (s, 2H), 3.70 – 3.51 (m, 2H), 2.09 – 1.92 (m, 4H), 1.86 (d, \(J = 11.2\) Hz, 2H), 1.67 (s, 4H), 1.60 – 1.36 (m, 8H), 1.32 (s, 4H), 1.21 (d, \(J = 11.2\) Hz, 12H), 1.09 – 0.87 (m, 22H), 0.82 – 0.61 (m, 12H); MS (ESI) \(m/z\) 351 (M + Na); HRMS (ESI) calcd for (C\(_{18}\)H\(_{36}\)NaO\(_3\)Si): 351.2326; found: 351.2325.
(3aR*,7aS*)-Hexahydrospiro[benzo[c][1,2]dioxole-3,1'-cyclohexane] (rac-11). To a stirred solution of 29 (70.0 mg, 0.21 mmol) and triethylamine (0.12 mL, 0.85 mmol) in dry DCM (2.0 mL), cooled to 0 °C, methanesulfonyl chloride (33.0 µL, 0.45 mmol) was added dropwise and the resulting mixture was stirred at 0 °C for 2 h. Thereafter, the organic phase was washed with water and brine, and dried over sodium sulfate. The organic solvent was removed under reduced pressure and the residue was purified by flash chromatography (1:40 ethyl acetate/petroleum ether 40-60) to afford rac-11 as white solid (31%); 1H NMR (300 MHz, CDCl₃) δ 4.55 – 4.45 (m, 1H), 2.39 – 2.27 (m, 1H), 2.04 – 1.90 (m, 1H), 1.83 – 1.53 (m, 10H), 1.51 – 1.34 (m, 7H); 13C NMR (75 MHz, CDCl₃) δ 86.8, 78.2, 52.0, 37.3, 31.0, 25.7 (2C), 24.4, 24.1, 24.0, 22.5, 20.3; MS (ESI) m/z 197 (M + H)+, 219 (M + Na)+; HRMS (ESI) calcd for (C₁₂H₂₀NaO₂)⁺: 219.1356; found: 219.1357. El. Anal. calcd for C₁₂H₂₀O₂: C, 73.43; H, 10.27; found: C, 73.29; H, 10.15.

(3R,S)-3-(1-Cyclohexenyl)-3-methylidihydrofuran-2(3H)-one (30). Starting from 16b, the title compound was prepared following the two-step procedure described for the synthesis of 13a and was obtained as white low melting solid (58% yield); 1H NMR (400 MHz, CDCl₃) δ 5.55 (s, 1H), 4.20 – 4.12 (m, 1H), 4.12 – 3.99 (m, 1H), 2.38 – 2.22 (m, 1H), 2.02 – 1.84 (m, 5H), 1.62 – 1.44 (m, 4H), 1.26 (s, 3H); MS (ESI) m/z 203 (M + Na)+; HRMS (ESI) calcd for (C₁₁H₁₆NaO₂)⁺: 203.1043; found: 203.1047.

(2R,S,3R,S)-3-(1-Cyclohexenyl)-2-methoxy-3-methyltetrahydrofuran (31). A mixture of 30 (0.20 g, 0.61 mmol) in DCM (2.0 mL), cooled to -78 °C, was treated with DIBAL (1.0 M in DCM, 0.91 mL, 0.91 mmol). After 30 min, the reaction mixture was quenched with ammonium chloride, the solid residue was filtered off and the aqueous phase was extracted with DCM. The crude 3-(1-cyclohexenyl)-3-methyltetrahydrofuran-2-ol (0.17 g, 0.91 mmol) was dissolved in methanol (1.0 mL) and a catalytic amount of p-toluensulfonic acid was added. The reaction mixture was stirred for 2 h at 25 °C, afterward a saturated solution of sodium bicarbonate was added, the volatiles were removed under reduced pressure and the aqueous phase was extracted with DCM. The organic phase was dried over sodium sulfate, the solvent was removed and the residue, obtained as a mixture of diastereoisomers (0.12 g, 69%) was directly used in the next step without further purification; 1H NMR (400 MHz, CDCl₃) δ 5.45 (s, 1H), 5.35 (s, 1H), 4.61 (s, 1H), 4.45 (s, 1H), 4.04 – 3.81 (m, 2H), 3.81 – 3.71 (m, 2H), 3.30 (s, 3H), 3.24 (s, 3H), 2.22 – 2.07 (m, 2H), 2.07 – 1.84 (s, 6H), 1.78 – 1.65 (m, 2H), 1.63 – 1.39 (m, 10H), 1.05 (s, 3H), 1.00 (s, 3H); MS (ESI) m/z 219 (M + Na)+; HRMS (ESI) calcd for (C₁₂H₂₀NaO₂)⁺: 219.1356; found: 219.1350.
Starting from 31, the title compound was prepared following the procedure described for the synthesis of 14a and was obtained as yellow oil (58% yield) and as a mixture of diastereoisomers; \(^1\)H NMR (400 MHz, CDCl\(_3\)) \(\delta\) 5.06 (s, 2H), 3.87 -3.66 (m, 4H), 3.31 (s, 6H), 2.31 (dd, \(J = 21.1, 9.8\) Hz, 2H), 1.87 (dd, \(J = 27.1, 14.0\) Hz, 4H), 1.63 – 1.36 (m, 12H), 1.31 – 1.16 (m, 6H), 1.00 (s, 6H), 0.93 (t, \(J = 4.8\) Hz, 18H), 0.65 (q, \(J = 7.9\) Hz, 12H); MS (ESI) \(m/z\) 367 (M + Na); HRMS (ESI) calcd for (C\(_{18}\)H\(_{36}\)NaO\(_4\)Si): 367.2275; found: 367.2273.

(3a'S,6a'R*)-3a'-Methyltetrahydrodrospiosiro[cyclohexane-1,3'-furo[2,3-c][1,2]dioxole] (rac-10). A mixture of 32 (0.12 g, 0.63 mmol) and trimethylsilyl trifluoromethanesulfonate (0.23 mL, 1.26 mmol) in DCM (2.0 mL) was stirred at -78 °C for 5 min. The reaction mixture was quenched by addition of a saturated solution of sodium bicarbonate, the layers were separated and the organic phase was washed with a saturated solution of sodium bicarbonate, dried over sodium sulfate and the solvent was removed. The crude product was purified by flash chromatography (1:10 ethyl acetate/petroleum ether 40-60) to afford the title compound (30% yield) as colorless oil; \(^1\)H NMR (400 MHz, CDCl\(_3\)) \(\delta\) 5.28 (s, 1H), 4.15 – 3.99 (m, 1H), 3.89 (dd, \(J = 13.4, 7.4\) Hz, 1H), 2.30 (dd, \(J = 15.6, 9.3\) Hz, 1H), 1.93 -1.84 (m, 1H), 1.72 – 1.56 (m, 3H), 1.53 – 1.36 (m, 5H), 1.28 – 1.19 (m, 1H), 1.16 (s, 3H), 1.11 – 1.01 (m, 1H); \(^13\)C NMR (75 MHz, CDCl\(_3\)) \(\delta\) 113.4, 87.6, 70.1, 65.4, 34.9, 31.2, 29.3, 25.8, 23.3, 21.8, 18.9; MS (ESI) \(m/z\) 221 (M + Na); HRMS (ESI) calcd for (C\(_{11}\)H\(_{18}\)NaO\(_3\))\(^+\): 221.1148; found: 221.1149. El. Anal. calcd for C\(_{11}\)H\(_{18}\)O\(_2\): C, 66.64; H, 9.15; found: C, 66.59; H, 9.41.

**Computational details**

All calculations performed in this work were carried out on Cooler Master Centurion 5 (Intel Core2 Quad CPU Q6600 @ 2.40 GHz) with Ubuntu 10.04 LTS (long-term support) operating system running Maestro 9.2 (Schrödinger, LLC, New York, NY, 2011)

**Molecules preparation.** The heme group was extracted from the protein data bank (PDB ID: 1CTJ) and used as initial structure in the preparation of free Fe(II)-heme, which is a planar molecule with a strong positive charge on its central iron atom, which lies slightly above the porphyrin plane. Accordingly, all parts of the pdb file were removed with the exclusion of porphyrin ring. Iron was charged as +2 and then prepared as reported in the next paragraph. All ligands were built using Maestro by means of build toolbar with the exception of compound 1 and artemisinin that were downloaded from PubChem (CID 10313564 and CID 68827) as .sdf file. The chirality of each ligand was specified during this step. All the compounds were treated by LigPrep, in order to obtain a starting structure to perform other
calculations. Conformational analyses were carried out by means of MacroModel application as previously reported.\textsuperscript{5}

**Ab Initio QM Calculations.** Bicyclic endoperoxides, compound 1 and artemisinin were treated using a protocol already described.\textsuperscript{6} Heme structure optimization was also performed by Jaguar, using B3LYP hybrid density functional method in combination with the LACVP* basis set, which is composed of 6-31G* description for all light atoms and of LANL2DZ for metal center such as iron.\textsuperscript{7} LACVP basis set has been successfully used in calculation on metalloporphyrins and derivatives as well as for transition metal complexes.\textsuperscript{8-11} The calculations were performed taking into account the solvation effects. Solvation was included by solving the Poisson-Boltzmann equations with a realistic molecular surface (van der Waals radius plus solvent radius about each atom) using the Jaguar solvation model (PBF).\textsuperscript{12} We used a dielectric constant of 80.37 and a probe radius of 1.4 Å for water. The properties established were ESP charges. These results are taken into account for docking calculation performed by Glide (see next paragraph).

**Molecular Docking.** Molecular docking studies were carried out by means of Glide software.\textsuperscript{13} The heme and the ligands used were prepared as reported in molecules preparation paragraph. The charges derived from QM calculation were considered for docking calculations by applying the OPLS_2005 force field. In fact, the mentioned force field allows a proper treatment of metals and has a wider range of atom types defined. The grid map for docking calculation was built taking into account the center of the iron atom with restricted grid to the length of the ligands used in the docking studies for establishing its size. Moreover, a metal charged acceptor constraint was introduced using symmetry option. After grid generation, the ligands were docked by using Glide standard precision (SP) mode. 1000 poses per docking run were written in the initial stage and 10 poses per ligand were selected for energy minimization. The 5 lowest energy poses obtained were subjected to post docking minimization. A single best conformation of each ligand was considered for the binding affinity analysis coupling GlideScore and visual inspection. Metal charged acceptor constraint were used. The distance between oxygen atoms and heme iron was calculated by measurement tool implemented in maestro, while the metal-coordination contacts were found by the same tool using contacts option.

**Prime/MM-GBSA simulation.** The Prime/MM-GBSA method implemented in Prime software\textsuperscript{14} consists in computing the change between the free and the complex state of both the ligand and the protein after energy minimization. The technique was applied using the
docking complexes obtained by means of Glide. The software was used to calculate the free-binding energy ($\Delta G_{\text{bind}}$) of each ligand, as recently reported by us.\textsuperscript{15,16}

**In vitro assays against P. falciparum strains**

Biological tests have been done on *in vitro* cultured *P. falciparum* parasites. *P. falciparum* parasites were cultured according to Trager and Jensen\textsuperscript{17} with slight modifications.\textsuperscript{6} The CQ-sensitive (D10) and the CQ-resistant (W2) strains were maintained at 5% hematocrit (human type A-positive red blood cells) in RPMI 1640 (EuroClone,) medium supplemented with 1% AlbuMaxII (lipid-rich bovine serum albumin) (Invitrogen), 0.01% hypoxantine (Sigma), 20 mM Hepes (EuroClone), 2 mM glutamine (EuroClone). All the cultures were maintained at 37 °C in a standard gas mixture consisting of 1% O\textsubscript{2}, 5% CO\textsubscript{2}, 94% N\textsubscript{2}.

The tested compounds and reference drug (CQ) were dissolved in either water or DMSO to a concentration of 10 mg/ml. Drugs were placed in 96-wells flat-bottomed microplates (Costar) and serial dilutions made with medium to achieve the required concentrations (final concentration ranging from 39.06 to 5000 ng/mL for compounds and final DMSO concentration ≤1%, which is non-toxic to the parasite). Asynchronous cultures with parasitaemia of 1–1.5% and 1% final hematocrit were aliquoted into the plates and incubated for 72 h at 37 °C. The parasites growth was determined spectrophotometrically (OD\textsubscript{650}) by measuring the activity of the parasite lactate dehydrogenase (pLDH), according to a modified version of Makler’s method in control and drug-treated cultures.\textsuperscript{18} Antimalarial activity was determined as concentration of drugs inducing 50% of growth inhibition (IC\textsubscript{50}). Each IC\textsubscript{50} value is the mean and standard deviation of at least three independent experiments performed in duplicate.

**Toxicity Evaluation.**

**Materials.** Dulbecco’s Modified Eagle’s Medium (DMEM), trypsin solution, and all the solvents used for cell culture were purchased from Lonza (Switzerland). Mouse immortalized fibroblasts NIH3T3 were purchased from American Type Culture Collection (Manassas, VA).

**Cell Cultures and Cytotoxicity Assay.** NIH3T3 were maintained in DMEM at 37 °C in a humidified atmosphere containing 5% CO\textsubscript{2}. The culture media were supplemented with 10% fetal calf serum (FCS), 1% L-glutamine–penicillin–streptomycin solution, and 1% MEM nonessential amino acid solution. Once at confluence, cells were washed with 0.1 M PBS, taken up with trypsin– EDTA solution, and then centrifuged at 1000 rpm for 5 min. The pellet was resuspended in medium solution (dilution 1:15). Cell viability after 24 h of incubation
with the compound was evaluated by Neutral Red Uptake (Sigma-Aldrich, Switzerland) by the procedure previously reported.\textsuperscript{19} Data are expressed as mean ± s.d. of three experiment repeated in six replicate. The data processing included the Student’s t test with p < 0.05 taken as significance level.

**In vivo assessment of anti-plasmodial activity**

*In vivo* activity of spiroperoxide \textbf{8} against erythrocytic stage development was assessed by the Peter’s 4 days test\textsuperscript{20} using the murine parasite *Plasmodium berghei* (ANKA strain, chloroquin sensitive) and 4 to 6 weeks old BALB/c female mice reared in the animal facilities of the University of Camerino. Rearing and handling of experimental animals was in compliance with the Italian Legislative Decree on the “protection of animals used for experimental and other scientific purposes” (D. Lgs. of 01/27/92) and in full adherence with the European Directive 2010/63/UE. Approval for conduct of the experiment has been obtained by the ethical committee of the University of Camerino.

Fourteen mice were infected intraperitoneally (i.p.) with \(10^7\) red blood cells positive for *P. berghei* parasites. The same day, the animals (= 7) allocated to the treatment group received spiroperoxide \textbf{8} at a dosage of 100 mg/kg dissolved in olive oil by the i.p. route (200 µL inoculum). Control mice were treated with 200 µL of olive oil solvent. Impact on parasite proliferation was assessed on day 5 of the experiment after 4 days of treatment. Thin smears were prepared from drops of tail blood and stained with 7.5% Giemsa solution (azar eosin methylene blue solution for microscopy, Merck, Germany). Smears were examined under the microscope (100x objective) and numbers of erythrocytes infected with asexual and sexual parasites enumerated over at least 300 red blood cells on 3 different optical fields. Mean percent parasitaemia and Cl\textsubscript{95} values were calculated for treatment and control groups.
NMR spectra of key compounds
18a (alcohol precursor)
30 (alcohol precursor)
References