

# Second Generation Analogues of RKA 182: Synthetic Tetraoxanes with outstanding *In Vitro* and *In Vivo* Antimalarial Activities

## Supporting Information

### Experimental

#### Chemistry

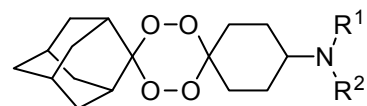
Air- and moisture-sensitive reactions were carried out in oven-dried glassware sealed with rubber septa under a positive pressure of dry nitrogen or argon from a manifold or balloon, unless otherwise indicated. Similarly sensitive liquids and solutions were transferred via syringe or stainless steel cannula. Reactions were stirred using Teflon-coated magnetic stir bars. Organic solutions were concentrated using a Buchi rotary evaporator with a diaphragm vacuum pump.

Anhydrous solvents were either obtained from commercial sources or dried and distilled immediately prior to use under a constant flow of dry nitrogen. THF and diethyl ether were distilled from Na, CH<sub>2</sub>Cl<sub>2</sub> and from CaH<sub>2</sub>. All other reagents were used as received from commercial sources unless otherwise indicated.

Analytical thin layer chromatography was performed with 0.25 mm Silica Gel 60F plates with 254 nm fluorescent indicator from Merck. Plates were visualised by ultraviolet light or by treatment with iodine, *p*-anisaldehyde, ninhydrin or potassium permanganate followed by gentle heating. Chromatographic purification of products was accomplished by flash chromatography on silica gel, as described by Still et al.

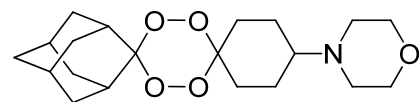
Melting points were determined on a Gallenkamp apparatus and are uncorrected. NMR spectra were measured on Bruker (400 MHz) nuclear magnetic resonance spectrometer. Solvents are indicated in the text. Data for <sup>1</sup>H NMR spectra are reported as follows: chemical shift ( $\delta$ , ppm) relative to tetramethylsilane as the internal reference, multiplicity (s = singlet, br s = broad singlet, d = doublet, t = triplet, q = quartet, sex = sextet, td = triplet of doublets, m = multiplet), coupling constant (*J*, Hz), integration, assignment. Data for <sup>13</sup>C NMR are reported in terms of chemical shift ( $\delta$ , ppm) relative to residual solvent peak. Mass spectra (MS) and high resolution mass spectra (HRMS) were recorded on a Micromass LCT mass spectrometer (ESI). Reported mass values are within error limits of  $\pm 5$  ppm. Elemental analyses (%C, %H, %N) were determined by the University of Liverpool Microanalysis Laboratory. Reported atomic percentages are within error limits of  $\pm 0.5\%$ .

#### General procedure for the reductive amination reaction



To a solution of compound **7** (1 eq., 0.05M) in anhydrous CH<sub>2</sub>Cl<sub>2</sub> was added the respective amine (1.5 eq.) and the mixture was allowed to stir at room temperature for 30 minutes followed by the addition of sodium triacetoxyborohydride (1.5 eq.). After stirring at room temperature for 16 hours, saturated aq. NaHCO<sub>3</sub> was added and the aqueous phase was extracted with CH<sub>2</sub>Cl<sub>2</sub>. The combined organic extracts were washed with saturated aq. NaCl, dried over MgSO<sub>4</sub>, filtered and concentrated *in vacuo*. The product was purified by flash chromatography on silica gel followed by recrystallisation if necessary.

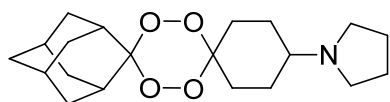
#### 4-(Dispiro[cyclohexane-1,3'-[1,2,4,5]tetroxane-6',2"-tricyclo[3.3.1.1<sup>3,7</sup>]decan]-4-yl)morpholine (**8**)



**8** was isolated as a pale yellow solid (52% yield) according to the general procedure utilising morpholine as the amine and following purification by flash chromatography on silica gel (100% EtOAc). <sup>1</sup>H NMR (400 MHz, CDCl<sub>3</sub>)  $\delta$  3.83 – 3.66 (m, 4H), 3.16 (s, 1H), 3.05 (s, 1H), 2.66 – 2.50 (m, 4H), 2.36 (t, *J* = 9.8 Hz, 1H), 1.97 (br s, 4H), 1.85 (br s, 3H), 1.80 – 1.65 (m, 8H), 1.62 (s, 5H); <sup>13</sup>C NMR (100 MHz, CDCl<sub>3</sub>)  $\delta$  110.8, 107.9, 67.8, 62.5, 50.1, 37.3, 34.7, 33.5, 30.6, 28.2, 27.4, 24.2, 23.7. MS (ES+) [M + H]<sup>+</sup> 366.1 (100), HRMS calculated for C<sub>20</sub>H<sub>32</sub>NO<sub>5</sub> 366.2280, found 366.2295; Elemental analysis C: 65.69, H: 8.76, N: 3.75 (required values C: 65.73, H: 8.55, N: 3.83).

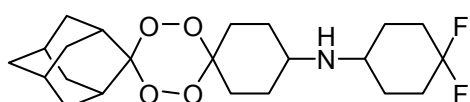
**1-(Dispiro[cyclohexane-1,3'-[1,2,4,5]tetroxane-6',2''-tricyclo[3.3.1.1<sup>3,7</sup>]decan]**

**-4-yl)pyrrolidine (9)**



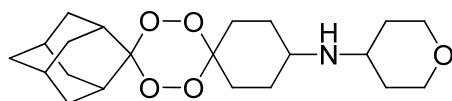
**9** was isolated as a pale yellow solid (66% yield) according to the general procedure utilising pyrrolidine as the amine and following purification by flash chromatography on silica gel (98:2 CH<sub>2</sub>Cl<sub>2</sub>/MeOH). <sup>1</sup>H NMR (400 MHz, CDCl<sub>3</sub>) δ 3.16 (br s, 1H), 3.01 (br s, 1H), 2.72 (br s, 4H), 2.38 (br s, 1H), 1.93 (br s, 6H), 1.85 (br s, 9H), 1.70 (br s, 6H), 1.67 – 1.55 (m, 3H); <sup>13</sup>C NMR (100 MHz, CDCl<sub>3</sub>) δ 110.8, 107.7, 62.0, 51.7, 37.3, 34.7, 33.5, 30.4, 30.1, 27.4, 26.9, 26.5, 23.9; MS (ES+) [M + H]<sup>+</sup> 350.2 (100), HRMS calculated for C<sub>20</sub>H<sub>32</sub>NO<sub>4</sub> 350.2331, found 350.2340; Elemental analysis C: 65.66, H: 8.77, N: 3.69 (required values C: 68.74, H: 8.94, N: 4.01).

**N-(4,4-Difluorocyclohexyl)dispiro[cyclohexane-1,3'-[1,2,4,5]tetroxane-6',2''-tricyclo[3.3.1.1<sup>3,7</sup>]decan]-4-amine (10)**



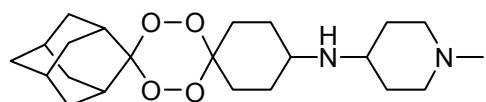
**10** was isolated as a white solid (73% yield) according to the general procedure utilising 4,4-difluorocyclohexylamine as the amine and following purification by flash chromatography on silica gel (99:1 CH<sub>2</sub>Cl<sub>2</sub>/MeOH). <sup>1</sup>H NMR (400 MHz, CDCl<sub>3</sub>) δ 3.16 (s, 1H), 2.96 (s, 1H), 2.80 – 2.64 (m, 2H), 2.17 – 2.03 (m, 3H), 2.03 – 1.91 (m, 4H), 1.87 (s, 7H), 1.82 – 1.67 (m, 8H), 1.63 (s, 2H), 1.54 – 1.29 (m, 5H); <sup>13</sup>C NMR (100 MHz, CDCl<sub>3</sub>) δ 123.7, 110.9, 108.0, 53.8, 52.3, 51.1, 37.3, 33.5, 32.2 (t, J = 24.4 Hz), 30.4, 29.8, 29.8, 28.9, 27.5; MS (ES+) [M + H]<sup>+</sup> 414.2 (100); HRMS calculated for C<sub>22</sub>H<sub>34</sub>F<sub>2</sub>NO<sub>4</sub> 414.2456, found 414.2458; Elemental analysis C: 63.49, H: 8.23, N: 3.31 (required values C: 63.90, H: 8.04, N: 3.39).

**N-(Tetrahydro-2H-pyran-4-yl)dispiro[cyclohexane-1,3'-[1,2,4,5]tetroxane-6',2''-tricyclo[3.3.1.1<sup>3,7</sup>]decan]-4-amine (11)**



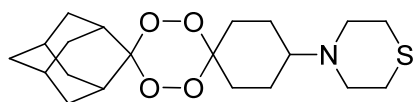
**11** was isolated as a white solid (84% yield) according to the general procedure utilising 4-aminotetrahydropyran as the amine and following purification by flash chromatography on silica gel (98:2 CH<sub>2</sub>Cl<sub>2</sub>/MeOH). <sup>1</sup>H NMR (400 MHz, CDCl<sub>3</sub>) δ 3.97 (d, J = 11.2 Hz, 2H), 3.39 (t, J = 11.2 Hz, 2H), 3.16 (br s, 1H), 2.97 (br s, 1H), 2.86 – 2.70 (m, 2H), 1.97 (br s, 5H), 1.87 (s, 5H), 1.81 (s, 2H), 1.78 (s, 2H), 1.71 (s, 4H), 1.62 (s, 2H), 1.50 – 1.31 (m, 5H); <sup>13</sup>C NMR (100 MHz, CDCl<sub>3</sub>) δ 110.8, 108.0, 67.3, 51.5, 51.0, 37.3, 34.7, 33.5, 30.5, 30.2, 29.4, 28.9, 27.8, 27.4; MS (ES+) [M + H]<sup>+</sup> 380.3 (100); HRMS calculated for C<sub>21</sub>H<sub>34</sub>NO<sub>5</sub> 380.2437, found 380.2452; Elemental analysis C: 66.12, H: 8.90, N: 3.62 (required values C: 66.46, H: 8.76, N: 3.69).

**N-(Dispiro[cyclohexane-1,3'-[1,2,4,5]tetroxane-6',2''-tricyclo[3.3.1.1<sup>3,7</sup>]decan]-4-yl)-1-methylpiperidin-4-amine (12)**



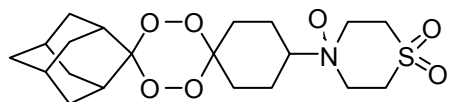
**12** was isolated as a white solid (19% yield) according to the general procedure utilising 1-methyl-4-aminopiperidine as the amine and following purification by flash chromatography on silica gel (95:5 CH<sub>2</sub>Cl<sub>2</sub>/MeOH). <sup>1</sup>H NMR (400 MHz, CDCl<sub>3</sub>) δ 3.16 (s, 1H), 2.95 (s, 1H), 2.83 (d, J = 11.7 Hz, 2H), 2.80 – 2.70 (m, 1H), 2.62 – 2.50 (m, 1H), 2.27 (s, 3H), 2.09 – 1.91 (m, 6H), 1.86 (br s, 8H), 1.70 (s, 6H), 1.62 (s, 2H), 1.46 – 1.32 (m, 5H); <sup>13</sup>C NMR (100 MHz, CDCl<sub>3</sub>) δ 110.8, 108.1, 55.2, 51.9, 51.5, 46.7, 37.3, 34.7, 33.8, 33.5, 30.4, 29.6, 29.0, 27.9, 27.5; MS (ES+) [M + H]<sup>+</sup> 393.2 (100); HRMS calculated for C<sub>22</sub>H<sub>37</sub>N<sub>2</sub>O<sub>4</sub> 393.2753, found 393.2753; Elemental analysis C: 67.53, H: 9.23, N: 7.22 (required values C: 67.32, H: 9.24, N: 7.14).

**1-(Dispiro[cyclohexane-1,3'-[1,2,4,5]tetroxane-6',2''-tricyclo[3.3.1.1<sup>3,7</sup>]decan]-4-yl)piperidin-4-amine (13a)**



**13a** isolated as a yellow foam (60% yield) according to the general procedure utilising thiomorpholine as the amine and following purification by flash chromatography on silica gel (99:1 CH<sub>2</sub>Cl<sub>2</sub>/MeOH). <sup>1</sup>H NMR (400 MHz, CDCl<sub>3</sub>) δ 3.88 (s, 1H), 3.16 (s, 1H), 2.68 (s, 1H), 2.26 – 1.79 (m, 18H), 1.79 – 1.53 (m, 10H); <sup>13</sup>C NMR (100 MHz, CDCl<sub>3</sub>) δ 110.9, 107.7, 68.2, 37.3, 34.7, 33.5, 30.7, 30.4, 29.9, 28.7, 27.4, 26.0; MS (ES+) [M + H]<sup>+</sup> 382.2 (100).

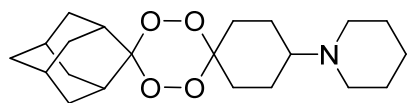
**4-(Dispiro[cyclohexane-1,3'-[1,2,4,5]tetroxane-6',2''-tricyclo[3.3.1.1<sup>3,7</sup>]decan]-4-yl)thiomorpholine 1,1,4-trioxide (13)**



To a solution of **13a** (295 mg, 0.7732 mmol) in  $\text{CH}_2\text{Cl}_2$  (35 ml) at  $0^\circ\text{C}$ , was added meta-chloroperoxybenzoic acid (520 mg, 2.319 mmol) and the reaction mixture was stirred at  $0^\circ\text{C}$  for 30 minutes. After 30 minutes the reaction mixture was

allowed to warm to room temperature and stirred for 16 hours. The reaction mixture was poured into cold 5% aq.  $\text{K}_2\text{CO}_3$  and extracted with  $\text{CH}_2\text{Cl}_2$ . The combined organic extracts were dried over  $\text{MgSO}_4$ , filtered and concentrated *in vacuo*. **13** (162 mg, 51%) was obtained as a white solid following purification by flash chromatography on silica gel (94:6  $\text{CH}_2\text{Cl}_2/\text{MeOH}$ ).  $^1\text{H}$  NMR (400 MHz,  $\text{CDCl}_3$ )  $\delta$  4.41 (s, 2H), 4.12 – 3.81 (m, 2H), 3.56 – 3.25 (m, 4H), 3.13 (s, 1H), 2.93 (d,  $J = 13.6$  Hz, 2H), 2.65 – 2.41 (m, 2H), 2.25 – 1.84 (m, 9H), 1.84 – 1.52 (m, 9H);  $^{13}\text{C}$  NMR (100 MHz,  $\text{CDCl}_3$ )  $\delta$  111.4, 106.5, 80.1, 61.1, 59.8, 53.9, 46.7, 37.2, 34.6, 33.5, 33.4, 30.9, 30.4, 28.6, 27.3, 23.2, 22.2; MS (ES+)  $[\text{M} + \text{Na}]^+$  452.1 (100); HRMS calculated for  $\text{C}_{20}\text{H}_{31}\text{NO}_7\text{SNa}$  452.1719, found 452.1714; Elemental analysis C: 54.50, H: 7.27, N: 3.26 (required values C: 55.93, H: 7.27, N: 3.26).

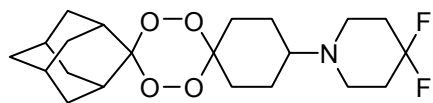
#### 1-(Dispiro[cyclohexane-1,3'-[1,2,4,5]tetroxane-6',2''-tricyclo[3.3.1.1<sup>3,7</sup>]decan]-4-yl)piperidine (**14**)



**14** was isolated as a white solid (27% yield) according to the general procedure utilising piperidine as the amine and following purification by flash chromatography on silica gel (97:3  $\text{CH}_2\text{Cl}_2/\text{MeOH}$ ).  $^1\text{H}$  NMR (400 MHz,  $\text{CDCl}_3$ )  $\delta$  3.16 (br s, 2H), 2.58 – 2.47 (m, 4H), 2.47 – 2.37 (m, 1H), 2.13 – 1.90 (m, 4H), 1.90 – 1.83 (m, 2H), 1.71 (br s, 8H), 1.66 – 1.49 (m, 10H), 1.48 – 1.38 (m, 2H);

$^{13}\text{C}$  NMR (100 MHz,  $\text{CDCl}_3$ )  $\delta$  110.8, 108.0, 63.6, 50.6, 37.3, 34.7, 33.5, 31.2, 30.5, 28.9, 27.4, 26.8, 25.1, 24.0, 23.4; MS (ES+)  $[\text{M} + \text{H}]^+$  364.3 (100); HRMS calculated  $\text{C}_{21}\text{H}_{34}\text{NO}_4$  364.2488 for, 364.2498 found; Elemental analysis C: 69.28, H: 9.13, N: 3.80 (required values C: 69.39, H: 9.15, N: 3.85).

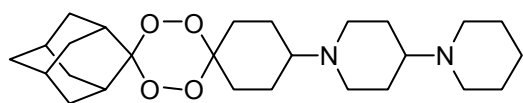
#### 1-(Dispiro[cyclohexane-1,3'-[1,2,4,5]tetroxane-6',2''-tricyclo[3.3.1.1<sup>3,7</sup>]decan]-4-yl)-4,4-difluoropiperidine (**15**)



**15** was isolated as a white solid (56% yield) according to the general procedure utilising 4,4-difluoropiperidine as the amine and following purification by flash chromatography on silica gel (9:1 *n*-Hex/EtOAc).  $^1\text{H}$  NMR (400 MHz,  $\text{CDCl}_3$ )  $\delta$  3.15

(br s, 2H), 2.79 – 2.60 (m, 4H), 2.59 – 2.45 (m, 1H), 2.19 – 1.91 (m, 10H), 1.87 (br s, 3H), 1.84 – 1.46 (m, 11H);  $^{13}\text{C}$  NMR (100 MHz,  $\text{CDCl}_3$ )  $\delta$  122.6 (t,  $J = 240$  Hz), 110.9, 107.8, 62.4, 46.2, 37.3, 34.9 (t,  $J = 23$  Hz), 33.5, 31.1, 30.5, 28.7, 27.4, 24.5, 23.9; MS (ES+)  $[\text{M} + \text{H}]^+$  400.3 (100); HRMS calculated for  $\text{C}_{21}\text{H}_{32}\text{F}_2\text{NO}_4$  400.2299, found 400.2284; Elemental analysis C: 62.91, H: 7.84, N: 4.54 (required values C: 63.14, H: 7.82, N: 3.51).

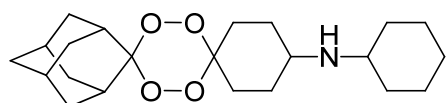
#### 1'-(Dispiro[cyclohexane-1,3'-[1,2,4,5]tetroxane-6',2''-tricyclo[3.3.1.1<sup>3,7</sup>]decan]-4-yl)-1,4'-bipiperidine (**16**)



**16** was isolated as a pale yellow solid (32% yield) according to the general procedure utilising 4-piperidinopiperidine as the amine and following purification by flash chromatography on silica gel (95:5  $\text{CH}_2\text{Cl}_2/\text{MeOH}$ ).  $^1\text{H}$

NMR (400 MHz,  $\text{CDCl}_3$ )  $\delta$  3.15 (br s, 2H), 2.93 (d,  $J = 10.8$  Hz, 2H), 2.51 (s, 4H), 2.47 – 2.38 (m, 1H), 2.34 – 2.11 (m, 3H), 1.96 (s, 4H), 1.87 (d,  $J = 2.0$  Hz, 3H), 1.83 – 1.67 (m, 9H), 1.67 – 1.47 (m, 12H), 1.43 (d,  $J = 4.9$  Hz, 2H);  $^{13}\text{C}$  NMR (100 MHz,  $\text{CDCl}_3$ )  $\delta$  110.7, 107.9, 63.5, 62.7, 50.5, 49.5, 37.3, 34.6, 33.5, 31.1, 30.4, 28.8, 28.5, 27.4, 26.8, 25.2, 24.3, 23.7; MS (ES+)  $[\text{M} + \text{H}]^+$  447.3 (100); HRMS calculated for  $\text{C}_{26}\text{H}_{43}\text{N}_2\text{O}_4$  447.3223, found 447.3205; Elemental analysis C: 69.82, H: 9.46, N: 6.24 (required values C: 69.92, H: 9.48, N: 6.27).

#### N-Cyclohexyldispiro[cyclohexane-1,3'-[1,2,4,5]tetroxane-6',2''-tricyclo[3.3.1.1<sup>3,7</sup>]decan]-4-amine (**17**)

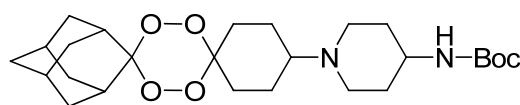


**17** was isolated as a white solid (58% yield) according to the general procedure utilising cyclohexylamine as the amine and following purification by flash chromatography on silica gel (98:2  $\text{CH}_2\text{Cl}_2/\text{MeOH}$ ) followed by recrystallisation in

hexane.  $^1\text{H}$  NMR (400 MHz,  $\text{CDCl}_3$ )  $\delta$  3.16 (br s, 1H), 2.98 (br s, 1H), 2.78 – 2.66 (m, 1H), 2.53 (tt,  $J = 10.5, 3.7$  Hz, 1H), 1.97 (br s, 4H), 1.86 (br s, 6H), 1.82 (br s, 2H), 1.79 – 1.66 (m, 8H), 1.66 – 1.55 (m, 3H), 1.36 (br s, 2H), 1.31 – 1.10 (m, 4H), 1.10 – 0.96 (m, 2H);  $^{13}\text{C}$  NMR (100 MHz,  $\text{CDCl}_3$ )  $\delta$  110.8, 108.1, 53.8, 52.0, 37.3, 34.5, 33.5, 30.5, 29.5, 29.0, 28.1, 27.5, 26.5, 25.6;

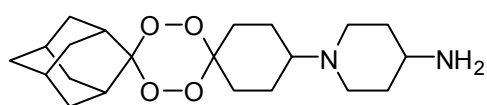
MS (ES+) [M + H]<sup>+</sup> 378.3 (100); HRMS calculated for C<sub>22</sub>H<sub>36</sub>NO<sub>4</sub> 378.2644, found 378.2643; Elemental analysis C: 69.55, H: 9.49, N: 3.58 (required values C: 69.99, H: 9.34, N: 3.71).

**Tert-butyl[1-(dispiro[cyclohexane-1,3'-[1,2,4,5]tetroxane-6',2''-tricyclo[3.3.1.1<sup>3,7</sup>]decan]-4-yl)piperidin-4-yl]carbamate (22)**



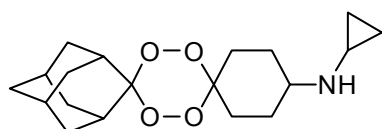
**22** was isolated as a white solid in 48% according to the general procedure utilising N-Boc-4-aminopiperidine as the amine and following purification by flash chromatography on silica gel (98:2 CH<sub>2</sub>Cl<sub>2</sub>/MeOH). <sup>1</sup>H NMR (400 MHz, CDCl<sub>3</sub>) δ 4.46 (d, *J* = 7.1 Hz, 1H), 3.45 (br s, 1H), 3.15 (br s, 1H), 2.93 - 2.79 (m, 2H), 2.55 - 2.40 (m, 1H), 2.33 (t, *J* = 10.7 Hz, 2H), 2.12 - 1.83 (m, 11H), 1.82 - 1.65 (m, 7H), 1.60 (br s, 6H), 1.44 (br s, 10H); <sup>13</sup>C NMR (100 MHz, CDCl<sub>3</sub>) δ 155.58, 110.88, 107.79, 79.67, 62.87, 48.43, 37.31, 34.65, 33.51, 33.27, 31.04, 30.43, 28.81, 27.41, 24.10, 23.59; MS (ES+) [M + H]<sup>+</sup> (100), 478.3; HRMS calculated for 477.2965 C<sub>21</sub>H<sub>35</sub>N<sub>2</sub>O<sub>4</sub>, found 477,2948; Elemental analysis C: 66.10, H: 8.74, N: 3.64 (required values C: 66.46, H: 8.76, N: 3.69).

**1-(Dispiro[cyclohexane-1,3'-[1,2,4,5]tetroxane-6',2''-tricyclo[3.3.1.1<sup>3,7</sup>]decan]-4-yl)piperidin-4-amine (18)**



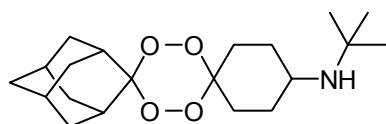
To a solution of **22** (250 mg, 0.52 mmol) in anhydrous CH<sub>2</sub>Cl<sub>2</sub> (8 ml) was added Amberlyst resin (500 mg) and the mixture stirred for 24 hours at room temperature. The mixture was filtered and the resin washed with CH<sub>2</sub>Cl<sub>2</sub>, *n*-Hex and MeOH. The washed resin was suspended in EtOH (2 ml) and treated with ammonia solution [2.0M in EtOH](2 ml). After stirring at room temperature for 1 hour, the resin was removed by filtration and the filtrate was concentrated to give **18** as a colourless oil. (130 mg, 66%). <sup>1</sup>H NMR (400 MHz, CDCl<sub>3</sub>) δ 3.03 (s, 2H), 2.71 (d, *J* = 11.4 Hz, 2H), 2.58 - 2.42 (m, 1H), 2.39 - 2.28 (m, 1H), 2.13 (td, *J* = 11.4, 2.1 Hz, 2H), 1.84 (s, 3H), 1.79 - 1.66 (m, 6H), 1.66 - 1.55 (m, 7H), 1.49 (s, 5H), 1.29 - 1.16 (m, 3H), 1.13 (s, 2H); <sup>13</sup>C NMR (100 MHz, CDCl<sub>3</sub>) δ 110.8, 107.9, 62.8, 49.5, 48.5, 37.3, 36.8, 34.7, 33.5, 31.2, 30.5, 28.8, 27.4, 24.2, 23.8; MS (ES+) [M + H]<sup>+</sup> 379.2 (100); HRMS calculated for C<sub>21</sub>H<sub>35</sub>N<sub>2</sub>O<sub>4</sub> 379.2597, found 379.2588; Elemental analysis C: 66.00, H: 8.95, N: 6.64 (required values C: 66.64, H: 9.05, N: 7.04).

**N-cyclopropylidisp[ro[cyclohexane-1,3'-[1,2,4,5]tetroxane-6',2''-tricyclo[3.3.1.1<sup>3,7</sup>]decan]-4-amine (19)**



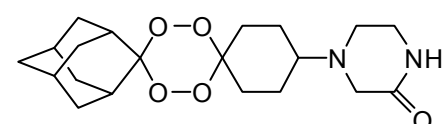
**19** was isolated as a white solid (86% yield) according to the general procedure utilising cyclopropylamine as the amine and following purification by flash chromatography on silica gel (99:1 CH<sub>2</sub>Cl<sub>2</sub>/MeOH). <sup>1</sup>H NMR (400 MHz, CDCl<sub>3</sub>) δ 3.16 (s, 1H), 2.96 (d, *J* = 11.1 Hz, 1H), 2.73 (tt, *J* = 9.8, 3.7 Hz, 1H), 2.19 - 2.09 (m, 1H), 2.06 - 1.84 (m, 9H), 1.84 - 1.66 (m, 6H), 1.66 - 1.31 (m, 6H), 0.50 - 0.41 (m, 2H), 0.36 - 0.28 (m, 2H); <sup>13</sup>C NMR (100 MHz, CDCl<sub>3</sub>) δ 110.8, 108.2, 56.0, 37.3, 34.7, 33.5, 30.5, 30.3, 29.3, 28.6, 28.6, 27.8, 27.4, 26.9; MS (ES+) [M + H]<sup>+</sup> 336.2 (100); HRMS calculated for C<sub>19</sub>H<sub>30</sub>NO<sub>4</sub> 336.2175, found 336.2173; Elemental analysis C: 67.79, H: 8.81, N: 4.09 (required values C: 68.03, H: 8.71, N: 4.18).

**N-tert-butylidisp[ro[cyclohexane-1,3'-[1,2,4,5]tetroxane-6',2''-tricyclo[3.3.1.1<sup>3,7</sup>]decan]-4-amine (20)**



**20** was isolated as a white solid (17% yield) according to the general procedure utilising *tert*-butylamine as the amine and following purification by flash chromatography on silica gel (99:1 CH<sub>2</sub>Cl<sub>2</sub>/MeOH). <sup>1</sup>H NMR (400 MHz, CDCl<sub>3</sub>) δ 3.15 (s, 1H), 3.01 (s, 1H), 2.75 - 2.62 (m, 1H), 2.10 - 1.89 (m, 4H), 1.83 (m, 3H), 1.83 - 1.65 (m, 8H), 1.65 - 1.36 (m, 6H), 1.15 (s, 9H); <sup>13</sup>C NMR (100 MHz, CDCl<sub>3</sub>) δ 110.8, 107.5, 52.3, 50.7, 37.3, 34.7, 33.5, 31.7, 31.2, 30.9, 30.4, 30.0, 28.5, 27.4; MS (ES+) [M + H]<sup>+</sup> 352.1 (100); HRMS calculated for C<sub>20</sub>H<sub>34</sub>NO<sub>4</sub> 352.2488, found 352.2490; Elemental analysis C: 67.82, H: 9.59, N: 3.99 (required values C: 68.34, H: 9.46, N: 3.99).

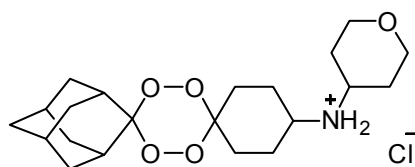
**4-(dispiro[cyclohexane-1,3'-[1,2,4,5]tetroxane-6',2''-tricyclo[3.3.1.1<sup>3,7</sup>]decan]-4-yl)piperazin-2-one (21)**



**21** was isolated as a white solid (92% yield) according to the general procedure utilising 2-oxopiperazine as the amine and following purification by flash

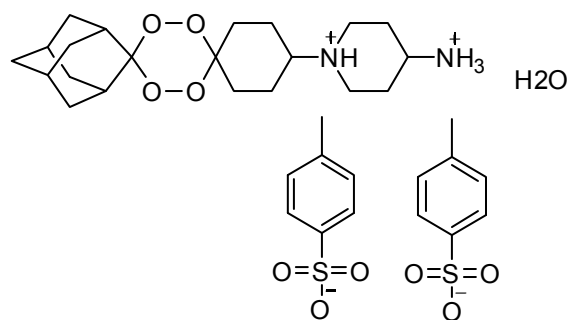
chromatography on silica gel (98:2 CH<sub>2</sub>Cl<sub>2</sub>/MeOH). <sup>1</sup>H NMR (400 MHz, CDCl<sub>3</sub>) δ 6.08 (s, 1H), 3.34 (td, *J* = 5.6, 2.2 Hz, 2H), 3.27 (s, 2H), 3.15 (s, 1H), 3.03 (s, 1H), 2.78 – 2.67 (t, *J* = 5.6 Hz, 2H), 2.52 – 2.41 (m, 1H), 2.08 – 1.91 (m, 4H), 1.91 – 1.84 (m, 3H), 1.84 – 1.68 (m, 7H), 1.68 – 1.42 (m, 6H); <sup>13</sup>C NMR (100 MHz, CDCl<sub>3</sub>) δ 170.1, 111.0, 107.7, 61.1, 54.2, 45.6, 42.3, 37.3, 33.5, 30.4, 27.4, 24.1; MS (ES+) [*M* + *H*]<sup>+</sup> 379.1 (100); [*M* + *Na*]<sup>+</sup> 401.1 (86); HRMS calculated for C<sub>20</sub>H<sub>31</sub>N<sub>2</sub>O<sub>5</sub> 379,2233, found 379,2234; calculated for C<sub>20</sub>H<sub>30</sub>N<sub>2</sub>O<sub>5</sub>Na 401.2052, found 401.2057; Elemental analysis C: 62.71, H: 7.96, N: 7.28 (required values C: 63.47, H: 7.99, N: 7.40).

***N*-(Tetrahydro-2*H*-pyran-4-yl)dispiro[cyclohexane-1,3'-[1,2,4,5]tetroxane-6',2''-tricyclo[3.3.1.1<sup>3,7</sup>]decan]-4-amine hydrochloride salt (23)**



**23** was isolated as a white solid (75% yield) by treating a solution of **11** in EtOH (0.5 ml) with hydrogen chloride [1.0M in Et<sub>2</sub>O] (1 ml). The mixture was heated at reflux for 20 minutes and once cooled a white precipitate crashed out which was filtered and washed with Et<sub>2</sub>O to give **23**. <sup>1</sup>H NMR (400 MHz, MeOH) δ 4.02 (dd, *J* = 11.8, 4.6 Hz, 2H), 3.54 – 3.41 (m, 4H), 3.41 – 3.34 (m, 1H), 3.28 – 3.20 (s, 1H (broad)), 3.13 (s, 1H (br)), 2.08 (s, 2H (br)), 2.04 – 1.88 (m, 6H), 1.88 – 1.52 (m, 15H); <sup>13</sup>C NMR (100 MHz, MeOH) δ 111.6, 107.3, 66.8, 52.6, 52.1, 37.8, 34.0, 33.9, 30.7, 28.4; MS (ES+) [*M* + *H*]<sup>+</sup> 380.2 (100); HRMS calculated for C<sub>21</sub>H<sub>34</sub>NO<sub>5</sub> 380.2437, found 380.2438; Elemental analysis C: 60.72, H: 8.19, N: 3.39 (required values C: 60.64, H: 8.24, N: 3.37).

**1-(Dispiro[cyclohexane-1,3'-[1,2,4,5]tetroxane-6',2''-tricyclo[3.3.1.1<sup>3,7</sup>]decan]-4-yl)piperidin-4-amine ditosylate salt monohydrate (24)**



To a solution of **22** (100 mg, 0.21 mmol) in MeOH (1 ml) was added *p*-toluenesulfonic acid monohydrate (87 mg, 0.46 mmol) and the reaction mixture was heated to 50 °C for 20 minutes. After cooling to room temperature a white precipitate formed which was filtered and washed with Et<sub>2</sub>O to give **24** (58 mg, 38%). <sup>1</sup>H NMR (400 MHz, MeOD) δ 7.71 (d, *J* = 8.2 Hz, 4H), 7.26 (d, *J* = 7.9 Hz, 4H), 3.62 (d, *J* = 11.8 Hz, 2H), 3.55 – 3.42 (m, 1H), 3.41 – 3.32 (m, 1H), 3.27 – 3.05 (m, 4H), 2.37 (s, 6H), 2.29 (d, *J* = 13.6 Hz, 2H), 2.15 – 1.80 (m, 8H), 1.80 – 1.52 (m, 14H); <sup>13</sup>C NMR (100 MHz, MeOH) δ 143.2, 141.9, 129.9, 126.9, 111.7, 107.0, 65.3, 46.8, 37.8, 35.6, 34.1, 34.0, 31.4, 30.5, 28.7, 28.5, 23.7, 23.1, 21.3; MS (ES+) [*M* + *H*]<sup>+</sup> 379.2 (100); HRMS calculated for C<sub>21</sub>H<sub>35</sub>N<sub>2</sub>O<sub>4</sub> 379.2597, found 379.2600; Elemental analysis C: 56.33, H: 7.01, N: 3.96 (required values C: 56.74, H: 7.07, N: 3.78).

***In vitro* Antimalarial Screening**

*Plasmodium falciparum* drug-sensitive 3D7 and chloroquine-resistant K1 strains were cultivated in a variation of the medium previously described<sup>[1,2]</sup>, consisting of RPMI 1640 supplemented with 0.5% ALBUMAX® II, 25 mM Hepes, 25 mM NaHCO<sub>3</sub> (pH 7.3), 0.36 mM hypoxanthine, and 100 µg/ml neomycin. Human erythrocytes served as host cells. Cultures were maintained in an atmosphere of 3% O<sub>2</sub>, 4% CO<sub>2</sub>, and 93% N<sub>2</sub> in humidified modular chambers at 37 °C. Compounds were dissolved in DMSO (10 mM), diluted in hypoxanthine-free culture medium and titrated in duplicates over a 64-fold range in 96 well plates. Infected erythrocytes (1.25% final hematocrit and 0.3% final parasitemia) were added into the wells. After 48 h incubation, 0.5 µCi of [<sup>3</sup>H]hypoxanthine was added per well and plates were incubated for an additional 24 h. Parasites were harvested onto glass-fiber filters and radioactivity was counted using a Betaplate liquid scintillation counter (Wallac, Zurich). The results were recorded and expressed as a percentage of the untreated controls. Fifty percent inhibitory concentrations (IC<sub>50</sub>) were estimated by linear interpolation.<sup>[3]</sup>

[1] A. Dorn, R. Stoffel, H. Matile, A. Bubendorf and R.G. Ridley, Malarial haemozoin/beta-haematin supports haem polymerization in the absence of protein, *Nature* **374** (1995), pp. 269–271.

[2] W. Trager and J.B. Jensen, Human malaria parasites in continuous culture, *Science* **20** (1976), pp. 673–675.

[3] W. Huber and J. Koella, A comparison of three methods of estimating EC<sub>50</sub> in studies of drug resistance of malaria parasites, *Acta Tropica* **55** (1993), pp. 257–261.

***In vivo* Antimalarial Screening**

In vivo  $1 \times 30$  mg/kg curative data reported in Table 2 and Table 3 was generated at the London School of Hygiene and Tropical Medicine.

#### Full suppressive 4-day Peters' test

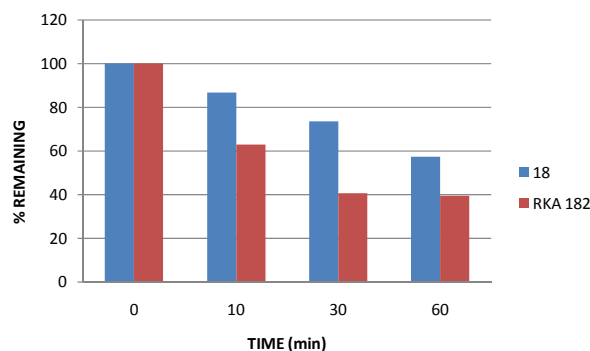
- For oral administration, compounds are dissolved in standard suspending formula (SSV) [0.5% sodium carboxymethylcellulose, 0.5% benzyl alcohol, 0.4% Tween 80, 0.9% NaCl (all Sigma)].
- Mice are infected intravenously with  $4 \times 10^6$  infected red cells from a donor mouse (day 0).
- Oral (p.o.) treatment is done with 0.2 ml of a solution of the test compounds 2-3 hours post-infection and on days 1, 2 and 3.
- Parasitaemia is determined by microscopic examination of Giemsa stained blood films taken on day 4 post infection.
- Microscopic counts of blood films from each mouse are processed using MICROSOFT® EXCEL spreadsheet (Microsoft Corp.), and expressed as percentages of inhibition from the arithmetic mean parasitaemias of each group in relation to the untreated group.

#### In vitro Metabolic Stability

Compounds were incubated with human liver microsomes. Incubation time points were made at 0, 10, 30 and 60 min with  $n=3$  at each time point. Drug incubations were done at  $10\mu\text{g ml}^{-1}$  with  $n=3$  at each concentration. Incubations were conducted in bigger volumes (1000  $\mu\text{L}$ ) and aliquots (200  $\mu\text{L}$ ) of samples were taken out at different time points. The microsomal protein content was  $1\text{ mg ml}^{-1}$ . NADPH concentration was  $1\text{ mg ml}^{-1}$ . Reaction was stopped by addition of 1:1 ice cold acetonitrile mixture. Samples were analysed by LC/MS/MS MRM method.

#### Incubation Results:

Time (min)	18		RKA182	
	% REMAINING	STDV	% REMAINING	STDV
0	100.00	0.00	100.00	0.00
10	86.74	2.47	62.91	17.89
30	73.62	8.02	40.56	5.75
60	57.41	4.59	39.53	13.99



- Results are expressed as % of remaining drug concentration at different time points.
- STDV: standard deviation values ( $n=3$ ).

#### Molecular Dynamics Simulations.

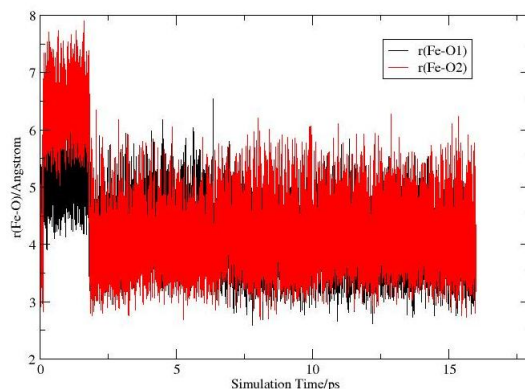


Figure Sx. A plot of interatomic distances  $r(\text{Fe-O1})$  and  $r(\text{Fe-O2})$  (the two tetraoxy oxygen atoms most proximal to the heme in Figure 3, main text) over course of the 16ps heating, equilibration and production simulation phases of the simulation. This peroxide bond was found to reach a stable distance from the  $\text{Fe}^{\text{II}}$  centre after  $\sim 2\text{ns}$ , with Fe-O interatomic distances then dynamically fluctuating between 3Å and 6Å.

All simulations were performed using the AMBER 11 molecular dynamics package.[1] Tetraoxane 8 was modelled using the general amber forcefield (GAFF) [2] with the tertiary amine group of the morpholine group being protonated. Heme was modelled using an adapted version of the ff99SB forcefield [3] modified with previously defined parameters specifically for Heme Fell [4,5]. Atomic charges for Tetraoxane 8 was obtained using Hartree-Fock ab initio quantum mechanical calculations.[6] All quantum calculations were performed using the locally dense 6-31G\* basis set as used in previous molecular mechanics parameterisation studies. [7] Electrostatic potential calculations were performed on geometries optimised at the same level of theory as used to calculate charge. Geometry optimisation and electrostatic potential calculations were performed using the SPARTAN '08 version 1.0.0 [8] similar to a previous parameterisation study in the literature. [7]

Prior to molecular dynamics simulations each system was minimised using the sander module in AMBER 11. The minimisation protocol used the steepest descent for 10000 steps followed by the conjugate gradient method for 10000 steps. Simulations were performed using a non-bonded cutoff of 12Å, temperature was controlled by Langevin dynamics with a collision frequency of 1 ps<sup>-1</sup>. The leapfrog algorithm was used to propagate the system, with a time step of 0.001 ps. A total of 16 ns of simulation was performed. Initially the system underwent a heating phase of 1 ns, followed by an 2 ns equilibration phase. The production phase of each simulation was 13 ns in duration.

### Supporting Information References

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