The artemiside-artemisox-artemisone-M1 tetrad: efficacies against blood stage *P. falciparum* parasites, DMPK properties, and the case for artemiside.

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**Supplementary Material**

**S1. Efficacy of artemisox**: Dose-response curves of artemisox **6** against asexual blood stages and gametocytes of *Plasmodium falciparum* (*Pf*).

**Fig. S1a**:Dose response curve of artemisox against the asexual blood stages of *Pf* NF54. The curve represents three independent biological repeats (n=3), ± SEM.

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**Fig. S1b**:Dose response curve of artemisox against the asexual blood stages of *Pf* K1. The curve represents three independent biological repeats (n=3), ± SEM.

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**Fig. S1c**: Dose response curve of artemisox against the asexual blood stages of *Pf* W2. The curve represents three independent biological repeats (n=3), ± SEM.

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**Fig. S1d**:Dose response curve of artemisox against early stage *Pf* NF54 gametocytes determined with the luciferase assay. Dose response was obtained at 48 h incubation. Each curve represents three independent biological repeats (n=3), ± SEM**.**

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**Fig. S1e**: Dose response curve of artemisox against late stage *Pf* NF54 gametocytes determined with the luciferase assay. Dose response was obtained at 72 h. Each curve represents three independent biological repeats (n=3), ± SEM.

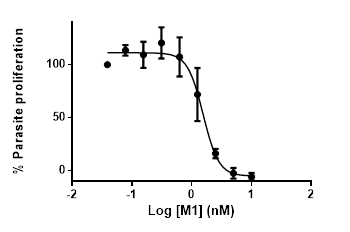
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**S2. Efficacy of M1**: Dose-response curves of M1 **8** against asexual blood stages and gametocytes of *Plasmodium falciparum*.

**Fig. S2a**:Dose response curve of M1 against the asexual blood stages of *Pf* NF54. The curve represents three independent biological repeats (n=3), ± SEM.



**Fig. S2b**:Dose response curve of M1 against the asexual blood stages of *Pf* K1. The curve represents three independent biological repeats (n=3), ± SEM.



**Fig. S2c**: Dose response curve of M1 against the asexual blood stages of *Pf* W2. The curve represents three independent biological repeats (n=3), ± SEM.



**Fig. S2d**:Dose response curve of M1 against early stage *Pf* NF54 gametocytes determined with the luciferase assay. Dose response was obtained at 48 h incubation. Each curve represents three independent biological repeats (n=3), ± SEM**.**



**S3. Pharmacokinetics and Metabolism**:Circulating concentrations of each of artemiside **5**, artemisox **6** and artemisone **7** and respective metabolites (µM) after iv and po dosing of parent compounds in mice; iv = intravenous, po = oral; sd = standard deviation, blq = below limit of quantitation; n/a not available; n = no. of mice used.

**Dosing of Artemiside****5**

**Table S3a**: Concentrations of artemiside **5**

|  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- |
| Time (h) | Mean iv (µM) | sd | n | Mean po (µM) | sd | n |
| 0.08 | 6.241 | 0.914 | 3 | 0.085 | 0.027 | 3 |
| 0.25 | 3.555 | 1.020 | 3 | 0.109 | 0.004 | 3 |
| 0.5 | 1.711 | 0.348 | 3 | 0.135 | 0.014 | 3 |
| 1 | 0.930 | 0.281 | 3 | 0.119 | 0.008 | 3 |
| 3 | 0.180 | 0.088 | 3 | 0.058 | 0.023 | 3 |
| 5 | 0.086 | 0.036 | 3 | 0.012 | 0.002 | 3 |
| 7 | 0.037 | 0.014 | 3 | 0.006 | 0.001 | 2 |
| 24 | 0.008 | 0.003 | 3 | blq | n/a | n/a |

**Table S3b**: Concentrations of artemisox **6** afterdosing of artemiside **5**

|  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- |
| Time (h) | Mean iv (µM) | sd | n | Mean po (µM) | sd | n |
| 0.08 | 1.926 | 0.400 | 3 | 1.864 | 0.260 | 3 |
| 0.25 | 1.817 | 0.400 | 3 | 2.150 | 0.382 | 3 |
| 0.5 | 1.376 | 0.339 | 3 | 2.510 | 0.282 | 3 |
| 1 | 0.674 | 0.388 | 3 | 2.026 | 0.183 | 3 |
| 3 | 0.066 | 0.017 | 3 | 0.286 | 0.228 | 3 |
| 5 | 0.020 | 0.001 | 3 | 0.007 | 0.001 | 3 |
| 7 | 0.011 | 0.004 | 3 | 0.005 | 0.000 | 1 |
| 24 | 0.005 | 0.000 | 2 | blq | n/a | n/a |

**Table S3c**: Concentrations of artemisone **7** afterdosing of artemiside **5**

|  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- |
| Time (h) | Mean iv (µM) | sd | n | Mean po (µM) | sd | n |
| 0.08 | 0.117 | 0.015 | 3 | 1.003 | 0.051 | 3 |
| 0.25 | 0.247 | 0.068 | 3 | 1.387 | 0.177 | 3 |
| 0.5 | 0.270 | 0.093 | 3 | 1.786 | 0.135 | 3 |
| 1 | 0.160 | 0.102 | 3 | 1.671 | 0.104 | 3 |
| 3 | 0.012 | 0.007 | 3 | 0.179 | 0.137 | 3 |
| 5 | 0.005 | 0.000 | 1 | blq | n/a | n/a |
| 7 | blq | n/a | n/a | blq | n/a | n/a |
| 24 | blq | n/a | n/a | blq | n/a | n/a |

**Fig. S3a**:LC-MS/MS chromatogram indicating presence of M1 following dosing of artemiside.a



**a**As determined by LC-MS/MS analysis with an AB SCIEX 5500 QTRAP instrument coupled with an Agilent 1260 HPLC detection system measured on mass of molecular ion [M+ H]+ (Transition 400.2/163.1) (ref. S[[1]](#endnote-1)).

**Dosing of Artemisox** **6**

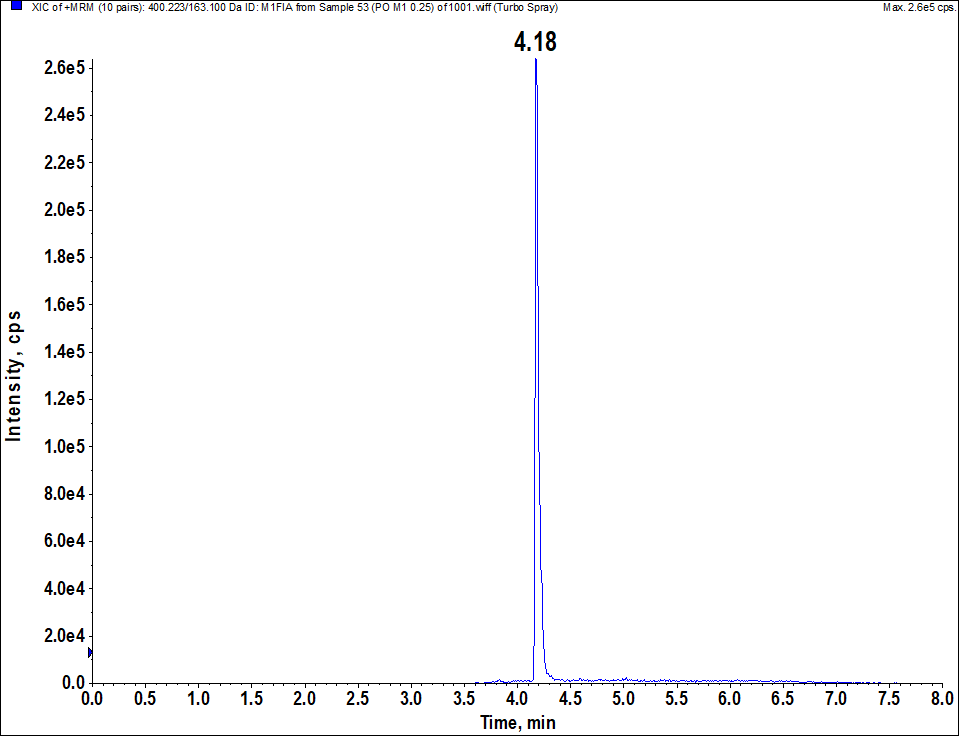
**Table S3d**: Concentrations of artemisox **6**

|  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- |
| Time (h) | Mean iv (µM) | sd | n | Mean po (µM) | sd | n |
| 0.08 | 1.897 | 1.181 | 3 | 3.043 | 0.512 | 3 |
| 0.25 | 2.420 | 0.537 | 3 | 4.207 | 2.670 | 3 |
| 0.5 | 1.920 | 0.608 | 3 | 2.153 | 0.344 | 3 |
| 1 | 0.663 | 0.266 | 3 | 2.013 | 1.616 | 3 |
| 3 | 0.007 | 0.003 | 3 | 0.013 | 0.006 | 3 |
| 5 | 0.007 | 0.003 | 2 | 0.005 | 0.004 | 3 |
| 7 | blq | n/a | n/a | 0.004 | 0.001 | 2 |
| 24 | blq | n/a | n/a | blq | n/a | n/a |

**Table S3e**: Concentrations of artemisone **7** after dosing of artemisox **6**

|  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- |
| Time (h) | Mean iv (µM) | sd | n | Mean po (µM) | sd | n |
| 0.08 | 1.112 | 0.716 | 3 | 3.643 | 0.328 | 3 |
| 0.25 | 1.404 | 0.138 | 3 | 3.534 | 0.997 | 3 |
| 0.5 | 1.250 | 0.181 | 3 | 3.686 | 0.792 | 3 |
| 1 | 0.659 | 0.164 | 3 | 3.202 | 0.156 | 3 |
| 3 | 0.007 | 0.000 | 1 | 0.048 | 0.018 | 3 |
| 5 | blq | n/a | n/a | 0.007 | 0.000 | 1 |
| 7 | blq | n/a | n/a | blq | n/a | n/a |
| 24 | blq | n/a | n/a | blq | n/a | n/a |

**Fig. S3b**:LC-MS/MS chromatogram indicating presence of M1 following dosing of artemisox.a



**a** As determined by LC-MS/MS analysis with an AB SCIEX 5500 QTRAP instrument coupled with an Agilent 1260 HPLC detection system measured on mass of molecular ion [M+ H]+ (transition 400.2/163.1) (ref.S1).

**Dosing of artemisone 7**

**Table S3f**: Concentration of artemisone **7**

|  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- |
| Time (h) | Mean iv (µM) | sd | n | Mean po (µM) | sd | n |
| 0.08 | 3.864 | 0.854 | 3 | 1.626 | 0.466 | 3 |
| 0.25 | 2.506 | 0.691 | 3 | 1.523 | 0.163 | 3 |
| 0.5 | 1.715 | 0.389 | 3 | 1.135 | 0.249 | 3 |
| 1 | 0.831 | 0.374 | 3 | 0.357 | 0.096 | 3 |
| 3 | 0.028 | 0.021 | 3 | 0.012 | 0.010 | 3 |
| 5 | blq | n/a | n/a | blq | n/a | n/a |
| 7 | blq | n/a | n/a | blq | n/a | n/a |
| 24 | blq | n/a | n/a | blq | n/a | n/a |

**Fig. S3c**:LC-MS/MS chromatogram indicating presence of M1 following dosing of artemisone.a



**a** As determined by LC-MS/MS analysis with an AB SCIEX 5500 QTRAP instrument coupled with an Agilent 1260 HPLC detection system measured on mass of molecular ion [M+ H]+ (transition 400.2/163.1) (ref. S1).

**S4. *In vitro* efficacy data (references** **S[[2]](#endnote-2),****S[[3]](#endnote-3))**

**Table S4a:** *In vitro* antimalarial activities for chloroquine, mefloquine, atovaquone, DHA **2**, artesunate **3**, artemisone **6**, metabolite M1 **12** determined with the [3H]-hypoxanthine drug susceptibility assay (ref. S2).a

|  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- |
| Compound | Antimalarial activities IC50 nM ± SEM | | | | | |
| D6 | W2 | 7G8 | TM93-C1088 | TM91-C235 | TM-C2B |
| Chloroquine CQ | 16 ± 2 | 195 ± 70 | 84 ± 18 | 360 ± 38 | 70 ± 12 | 95 ± 23 |
| Mefloquine MFQ | ND | ND | 5.4 ± 1.7 | 16 ± 5.0 | 107 ± 41 | 130 ± 51 |
| Atovaquone | ND | ND | 3.1±0.9 | 18,830 ± 5,102 | 2.2 ± 0.7 | 31,850 ± 6,833 |
| DHA **2** | 1.7 ± 0.4 | 2.2 ± 0.8 | 1.3 ± 0.2 | 1.4 ± 0.4 | 2.3 ± 0.7 | 2.0 ± 0.7 |
| Artesunate **4** | ND | 3.0 ± 1.6 | 1.5 ± 0.2 | 1.4 ± 0.1 | 2.9 ± 1.4 | 2.5 ± 0.5 |
| Artemisone **7** | 1.0 ± 0.4 | 1.3 ± 0.5 | 0.8 ± 0.1 | 0.7 ± 0.2 | 1.1 ± 0.5 | 1.1 ± 0.4 |
| Metabolite M1 **8** | 4.7 ± 0.2 | 6.6 ± 0.4 | 2.6 ± 0.8 | 2.5 ± 0.4 | 5.0 ± 0.6 | 8.6 ± 8.2 |

a D6 CQ sensitive; W2 and 7G8 CQ resistant; TM90-C2B and TM93-C1088 atovaquone and CQ resistant; TM91-C235 CQ and MFQ resistant; values represent the mean ± SD from three independent experiments carried out in triplicate

**Table S4b***: In vitro* antimalarial activities of amino-artemisinins against *P. falciparum* asexual blood stage artemisinin-resistant clones carrying the *Pf*KI3 C580Y mutation as determined with the [3H]-hypoxanthine drug susceptibility assay (ref. S3).

|  |  |  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- | --- | --- |
| Compound a | W2 | | ARC08-22 (4G) (48 h lc)b | | | PL08-009 (5C) (36 h lc)b | | |
| IC50 nM | IC90 nM | IC50 nM | IC90 nM | RIb | IC50 nM | IC90 nM | RIc |
| DHA **2** | 4.58±2.54 | 10.30±5.76 | 6.68±0.61 | 15.40±1.39 | 1.5 | 6.41±1.54 | 10.73±3.81 | 1.4 |
| Artemiside **5** | 2.21±0.42 | 4.28±0.25 | 2.43±0.13 | 4.85±0.09 | 1.1 | 0.29±0.03 | 0.43±0.03 | 0.1 |
| Artemisone **7** | 1.69±0.36 | 3.42±0.45 | 1.62±0.19 | 3.38±0.28 | 1.0 | 0.27±0.05 | 0.43±0.07 | 0.2 |

a Structures in Fig. 1; b lc = life cycle (h); c RI = IC50 for ARC08-22(4G)/IC50 forW2; d RI = IC50 for PL08-09 (5C)/IC50 forW2; Results are the mean of three independent biological replicates, performed as technical triplicates, ± SEM.

**Table S4c**: *In vitro* activities of selected amino-artemisinins against liver stage *P. berghei* sporozoites and cytotoxicities (ref. S3)a

|  |  |  |  |  |
| --- | --- | --- | --- | --- |
| Compound | IC50 nM | Maximum inhibition %  (Conc. µM) | Cytotoxicity EC50b | |
| *P. berghei* sporozoites | | HepG2 µM | SI |
| Atovaquone | 2.515±0.997 | 94.85±2.76 (0.5) | > 0.25 | > 100 |
| Puromycin | 22.7±4.525 | 110±4.24 (5) | 0.117 | 5.15 |
| Artemether **3** | >104 | 49.4 (10) | nd | nd |
| Artemiside **5** | 81.3±9.616 | 99.05±1.34 (5) | > 25.0 | > 308 |
| Artemisone **7** | 28.3±01.273 | 93.35±1.76 (10) | > 50.0 | > 1767 |

a Structures in Fig. 1; luciferase-expressing *P. berghei* ANKA GFP-Luc-SMcon sporozoites were allowed to invade HepG2 cells and luciferase activity was measured after 48 h; data ± SD from biological duplicate and technical quadruplicate measurements. SI = EC50 HepG2/IC50 *P. berghei* sporozoites

**S5. *In vivo* efficacy data from reference** **S[[4]](#endnote-4)**

**Table S5:** *In vivo* activities against CQ-sensitive *P. berghei* N strain and CQ-resistant *P. yoelii* NS strain(ref. S4).

|  |  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- | --- |
| Compound | *P. berghei* ED90 mg/kg | | *P. berghei* artesunate index | | *P. yoelii* ED90 mg/kg | | *P. yoelii* artesunate index |
| *sc* | *po* | *sc* | *po* | *sc* | *po* | *sc* |
| Artesunate **4** | 7.2 | 7.1 | 1.0 | 1.0 | 22.0 | - | 1.0 |
| Artemiside **5** | 0.51 | 1.9 | 14.1 | 4.9 | 0.61 | 2.0 | 36.0 |
| Artemisone **7** | 1.5 | 3.1 | 3.2 | 2.3 | 3.9 | 5.0 | 5.6 |

aPeters four-day test with mice treated daily subcutaneously (sc) or orally (po) from the day of infection (day 0) through day 3; ED90 values are evaluated from parasite counts in peripheral blood on day 4; bED90 artesunate/ ED90 compound.

**S6. *In vitro* neurotoxicity data from reference S4 according to methods from refs.** **S[[5]](#endnote-5),** **S[[6]](#endnote-6)**

**Table S6:** Effects of the test compounds on viability and neurofilaments in rat primary neuronal brain stem cell cultures.a

|  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- |
| Compound | Viability µg/mLb | | ATP µg/mLc | | Neurofilaments µg/mLd | |
| NOECe | IC50 | NOECe | IC50 | NOECe | IC50 |
| DHA **2** | 0.1 | 5.0 | 0.01 | 0.08 | <0.001 | 0.01 |
| Artesunate **4** | 0.1 | >10 | - | - | 0.1 | 5.0 |
| Artemiside **5** | 1.0 | >10 | 1.0 | 8.5 | 0.001 | >10 |
| Artemisone **7** | 10 | >10 | >10 | >10 | >10 | >10 |

aDetermined on fetal rat brain stem cells (E18–E19) cultured to generate a permanent neuronal network during days 1–8; compounds in dimethyl sulfoxide were applied at 0.001, 0.01, 0.1, 1, and 10 mg/mL for 7 days starting on day 9 according to refs. S5,S6. bCytotoxicity was measured by viability based on the activity of neuron-specific enolases. cATP (adenosine triphosphate) levels measured at 1 day after initial administration (refs 5,6); dNeurotoxicity was assessed by the effect on the cytoskeleton on day 7; eNo observable effect concentration.

**References**

1. S Watson, D.J., Laing, L., Gibhard, L., Wong, H.N., Haynes, R.K., Wiesner, L. Towards new transmission-blocking combination therapies - pharmacokinetics of 10-amino-artemisinins and 11-aza-artemisinin, and comparison with DHA and artemether. *Antimicrob. Agents Chemother*, **2021**, *65*, e00990-21, doi.10.1128/AAC.00990-21. [↑](#endnote-ref-1)
2. S Grobler, L., Chavchich, M., Haynes, R.K., Edstein, M.D., Grobler, A.F. Assessment of the induction of dormant ring stages in *Plasmodium falciparum* parasites by artemisone and artemisone entrapped in Pheroid vesicles *in vitro*. *Antimicrob. Agents Chemother.* **2014**, *58*,7579-7582, doi: 10.1128/AAC.02707-14. [↑](#endnote-ref-2)
3. S Wong, H.N., Padín-Irizarry, V., van der Watt, M.E., Reader, J., Liebenberg, W., Wiesner, L. et al. Optimal 10-aminoartemisinins with potent transmission-blocking capabilities for new artemisinin combination therapies – activities against blood stage *P. falciparum* Including *Pf*KI3 C580Y mutants and liver stage *P. berghei* parasites. *Front. Chem.* **2020**, *7*, 901,doi: 10.3389/fchem.2019.00901. [↑](#endnote-ref-3)
4. S Haynes, R.K., Fugmann, B., Stetter, J., Rieckmann, K., Heilmann, H.- D., Chan, H.-W. et al. Artemisone - a highly active antimalarial drug of the artemisinin class. *Angew. Chem. Internat. Edit.* **2006**,*45*, 2082-2088, doi: 10.1002/anie.200503071. [↑](#endnote-ref-4)
5. S Schmuck, G., Haynes, R.K. Establishment of an in vitro screening model for neuro­degeneration induced by antimalarial drugs of the artemisinin-type *Neurotoxic. Res.* **2000**, *2*, 37-49. doi: 10.1007/BF03033326. [↑](#endnote-ref-5)
6. S Schmuck, G., Roehrdanz, E., Haynes, R.K., Kahl, R. Neurotoxic mode of action of artemisinin. *Antimicrob. Agents Chemother.* **2002,** *46*, 821 – 827, doi: 10.1128/aac.46.3.821-827.2002. [↑](#endnote-ref-6)