Supplementary Material and Methods

PCR conditions and multiplex primer mixtures

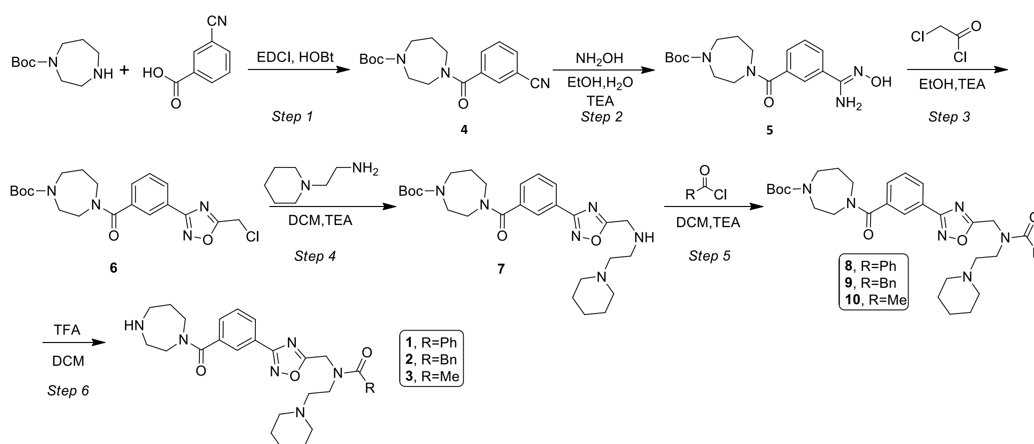
Four primary PCR reactions were set up with the following primer combinations (sequences are given in Table S1): Mix 1 (P1-1 for_P, P1_1 rev_P, P3-1 for_P, P3-1 rev_P, P8-1 for_P SL, P8-1 rev_P, P10-1 for_P, and P10-1 rev_P), Mix 2 (P5-1 for_P, P5-1 rev_P SL, P11-1 for_P, P11_1 rev_P , P12-1 for_P, P12-1 rev_P), Mix 3 (P18-1 for_P SL and P18-1 rev_P), and Mix 4 (P16-1 for_P SS and P16-1 rev_P SS). Nested PCR reactions were conducted individually as nP1 (P1 for_N and P1 rev_N), nP3 (P3 for_N SL and P3 rev_N), nP5 (P5 for_N and P5 rev_N SL), nP8 (P8 for_N and P8 rev_N SL), nP10 (P10 for_N and P10 rev_N SS), nP11 (P11 for_N and P11 rev_N), nP12 (P12 for_N and P12 rev_N), nP18 (P18 for_N and P18 rev_N), nP16 (P16 for_N and P16 rev_N SL). All reactions were run with the following program: denaturation for 3 min at 96°C followed by 30 cycles of 96°C for 30 sec, 52°C for 90 sec, and 72°C for 90 sec, except reaction Mix 3 and nP16 for which the annealing temperature was of 48°C instead of 52°C.

Synthesis and characterization of compounds 1, 2, 3

All reactions were carried out under nitrogen atmosphere or in sealed vials unless noted otherwise. Dry solvents and reagents were of commercial quality and were used as purchased. Reactions were magnetically stirred and monitored by thin-layer chromatography (TLC) using Merck silica gel 60 F254 by fluorescence quenching under UV light or by LCMS detection, except if indicated otherwise. LCMS-analyses were run on Agilent 1100/1200 series instruments. In addition, TLC plates were

24 stained using phosphomolybdic acid or potassium permanganate stain.

25 Chromatographic purification of products (flash chromatography) was performed on
26 Biotage Isolera systems and ethyl acetate/heptane gradients. Concentration under
27 reduced pressure was performed by rotary evaporation at 40°C at the appropriate
28 pressure unless otherwise stated. The purity of the compounds reported in the
29 manuscript was established through HPLC-MS methodology. HPLC analyses were
30 run according to the LCMS method. ^{1}H-NMR (in DMSO-D6) and mass spectra are in
31 agreement with the structures and were recorded on a Bruker AMX 400 MHz NMR
32 spectrometer (TMS as an internal standard). All the compounds reported in the
33 manuscript have a purity ≥ 95 % unless noted otherwise.



34

35 *Step 1.* To a solution of 4-cyano benzoic acid (4 g, 27 mmol) and *N*-Boc diazepene
36 (5.9 g, 29.9 mmol) in DCM (50 mL) triethylamine (11.3 mL, 81.5 mmol) was added
37 and stirred for 5 h. To this solution was added EDCI.HCl (6.7 g, 35.1 mmol) and
38 HOBt (0.73 g, 5.4 mmol) and stirred at RT overnight. The reaction mixture was
39 diluted with DCM, and washed with saturated sodium bicarbonate solution followed
40 by water and brine solution (NaCl sat aqueous). The combined organic layer was
41 dried on anhydrous Na_2SO_4 and solvents were removed by reduced pressure. The
42 crude material was purified by column chromatography over silica gel (230-400

43 mesh) using 15% ethyl acetate in petroleum ether as an eluent to afford the amide **4**

44 (4.5 g, 50%). *Step 2*. To a solution of compound **4** (3 g, 9.1 mmol) in EtOH:H_2O

45 (30:10 mL) mixture was added hydrazine hydrate (0.95 g, 13.6 mmol) and

46 triethylamine (3.16 mL, 22.7 mmol) and heated at 100°C for 3 h. After checking the

47 conversion of the starting material, the reaction mixture was cooled to RT and

48 concentrated under reduced pressure to yield compound **5** (5 g) as a crude white solid.

49 *Step 3*. To a solution of compound **5** (3 g, 8.28 mmol) in ethanol (15 mL)

50 triethylamine (1.72 mL, 12.4 mmol) was added and stirred at 0°C for 5 minutes. Then

51 chloro acetylchoride was added to the reaction mixture at 0°C and was heated at 80°C

52 in a sealed tube for 3 h. After checking the TLC, reaction mixture was cooled to RT,

53 and removed the solvent under reduced pressure. The residue obtained was dissolved

54 in DCM, washed with water, and the organic layer dried over anhydrous Na_2SO_4. The

55 crude material obtained was purified by column chromatography over silica gel (230-

56 400 mesh) using 90% of ethyl acetate in petroleum ether as an eluent to afford the

57 chloro intermediate **6** [0.83 g, 21% (combined yield step 2&3)] as a gummy liquid.

58 *Step 4*. To a solution of chloro intermediate **6** (0.83 g, 1.99 mmol) in DCM, was

59 added TEA (1.2 mL, 5.9 mmol) and stirred for 5 minutes, followed by drop wise

60 addition of 2-(piperidin-1-yl)ethanamine (0.38 g, 2.96 mmol) and the reaction mixture

61 was stirred at RT overnight. After completion of the starting material, the reaction

62 mixture was diluted with DCM and given a water wash followed by brine wash. The

63 combined organic layer was dried over anhydrous Na_2SO_4 and the solvent was

64 removed under reduced pressure to obtain a crude oil. The crude material was purified

65 by column chromatography over silica gel (230-400 mesh) using 4-6% of MeOH in

66 DCM as an eluent to afford compound **7** (0.8 g, 79%) as a brown liquid.

67 **N-((3-(3-(1,4-diazepane-1-carbonyl)phenyl)-1,2,4-oxadiazol-5-yl)methyl)-N-(2-**

68 **(piperidin-1-yl)ethyl)acetamide (3)**

69 *Step 5*. To a solution of compound **7** (0.25 g, 0.48 mmol) in DCM (10 mL), TEA (0.2

70 mL, 1.46 mmol) was added and allowed to stir for 5 minutes. The reaction mixture

71 was cooled to 0°C followed by the addition of acetyl chloride (0.057 g, 0.7 mmol) and

72 the whole warmed to RT and stirred for 30 minutes. The reaction mixture was diluted

73 with DCM and washed with water and brine. The organic layer was collected, dried

74 over anhydrous Na_2SO_4 and the solvent removed under reduced pressure. The crude

75 material was purified by column chromatography over silica gel (230-400 mesh)

76 using 4-6% of methanol (v/v) in DCM as an eluent to afford compound **8** (0.1 g, 40%)

77 as a brown liquid. *Step 6*. To a solution of compound **8** (0.1 g, 0.16 mmol) in DCM (3

78 mL), TFA (0.3 g, 2.4 mmol) was added at 0°C and stirred at room temperature (RT)

79 for 2 h. After completion of the reaction, the solvent mixture was removed under

80 reduced pressure. The residue obtained was dissolved in DCM and, to this, ion

81 exchange resin was added and stirred for 30 min. The resin was filtered off and the

82 filtrate concentrated to give **1** (0.07 g, 86%) as a gummy liquid. ^{1}H NMR, 400 MHz,

83 DMSO-d6: δ 8.03 (d, *J* = 7.40 Hz, 1H), 7.94 (s, 1H), 7.64-7.57 (m, 2H), 4.86 (s, 2H),

84 3.57 (s, 5H), 2.82 (t, *J* = 5.68 Hz, 3H), 2.38-2.32 (m, 5H), 2.13 (s, 3H), 1.70 (s, 2H),

85 1.43-1.26 (m, 6H).LC-MS APCI: Calculated for $C_{24}H_{34}N_6O_3$ 454.58; Observed m/z

86 [M+H]+ 455.4. Purity by LC-MS: 99.64%. Purity by HPLC: 99.78%.

87 **N-((3-(3-(1,4-diazepane-1-carbonyl)phenyl)-1,2,4-oxadiazol-5-yl)methyl)-N-(2-**

88 **(piperidin-1-yl)ethyl)benzamide (1)**

89 Yield: (50 mg, 60%).^{1}H NMR, 400 MHz, DMSO-d6: δ 8.07-8.05 (m, 1H), 7.97 (s,

90 1H), 7.66-7.58 (m, 2H), 7.48-7.44 (m, 5H), 5.02 (s, 2H), 3.67-3.46 (m, 4H), 2.84 (t, *J*

91 = 11.48 Hz, 4H), 2.32-2.24 (m, 4H), 1.73-1.71 (m, 2H), 1.43-1.39 (m, 4H), 1.33-1.27

92 (m, 3H). LC-MS APCI: Calculated for $C_{29}H_{36}N_6O_3$ 516.65; Observed m/z [M+H]+

93 517.4. Purity by LC-MS: 97.58%. Purity by HPLC: 99.79%.

94 **N-((3-(3-(1,4-diazepane-1-carbonyl)phenyl)-1,2,4-oxadiazol-5-yl)methyl)-2-**

95 **phenyl-N-(2-(piperidin-1-yl)ethyl)acetamide (2)**

96 Yield: (70 mg, 84%).^{1}H NMR, 400 MHz, DMSO-d6: δ 8.05-8.00 (m, 2H), 7.67-7.61

97 (m, 2H), 7.28-7.22 (m, 5H), 4.91 (s, 2H), 3.85 (s, 2H), 3.62-3.55 (m, 5H), 3.18-3.06

98 (m, 3H), 2.51-2.46 (m, 2H), 2.37-2.34 (m, 4H), 1.87 (s, 2H), 1.43-1.27 (m, 7H), 0.88

99 (t, J = 13.72 Hz, 3H). LC-MS APCI: Calculated for LC-MS APCI: Calculated for

100 $C_{29}H_{36}N_6O_3$ 530.68; Observed m/z [M+H]+ 531.4. Purity by LC-MS: 98.15%. Purity

101 by HPLC: 97.71%.

102

103 **Expression of HDP and falcipain 2**

104 The cultures of bacteria containing plasmid pHDP were grown to mid-exponential

105 phase and induced with isopropyl-1-thio-β-D-galactopyranoside (IPTG, 1 mM) for 4

106 h at 37°C and harvested by centrifugation at 4000 x g for 20 min. The total cell pellet

107 was then resuspended in wash buffer (50 mM Tris-HCl, pH 7.5, 20 mM EDTA) with

108 0.5 mg/ml lysozyme, incubated for 1 h at room temperature with intermittent shaking.

109 The washed cell pellet was lysed after adding wash buffer containing 0.5 M NaCl and

110 2.5% Triton X-100. The inclusion bodies were pelleted by centrifugation at 13,000

111 rpm for 50 min at 4°C, resuspended in wash buffer containing 1% Triton X-100 using

112 a sonicator, pelleted again, and then washed twice in wash buffer without Triton X-

113 100. The inclusion bodies were solubilized for 30 min in 50 mM CAPS buffer (pH

114 11.0) containing 1.5% N-lauryl sarcosine and 0.3 M NaCl and centrifuged at 10,000 x

115 g for 30 min. The protein was purified from the supernatant using a His-Trap, a high

116 performance nickel affinity column (GE Healthcare) by an imidazole gradient in 50
117 mM CAPS pH 11.0 containing 0.3% N-lauryl sarcosine and 0.3 M NaCl. Protein-
118 containing fractions were pooled and dialyzed against 25 mM CAPS buffer (pH 11.0)
119 containing 135 mM NaCl (30, 34).

120 For the expression of recombinant falcipain 2, bacteria containing plasmid pQE30-
121 FP2 were grown to mid-exponential phase and induced with 0.5 mM IPTG for 5 h at
122 37°C. The cells were harvested, washed with ice-cold 100 mM Tris-HCl pH 7.4, 10
123 mM EDTA buffer, sonicated (12 cycles of 10 s each, with cooling for 10 s between
124 the cycles), and centrifuged at 15,000 rpm for 45 min at 4°C. The pellet was
125 solubilized in 6 M guanidine HCl, 20 mM Tris-Cl, 250 mM NaCl, 20 mM imidazole,
126 pH 8.0 (5 ml/g of inclusion body pellet) at RT for 60 min with gentle stirring.
127 Insoluble material was separated by centrifuging at 15,000 rpm for 60 min at 4°C.
128 Recombinant protein was purified from the supernatant using a nickel-nitrilotriacetic
129 acid (Ni-NTA+) resin (Qiagen). The bound proteins were eluted with 1M imidazole in
130 20 mM Tris-HCl, pH 8.0 buffer containing 8M urea and quantified using Bradford
131 assay (Pierce).

132 For refolding of protein, the fractions containing falcipain 2 protein were pooled and
133 diluted 100-fold in ice-cold refolding buffer containing: 100 mM Tris-HCl pH 8.0, 1
134 mM EDTA, 20% glycerol (v/v), 250 mM L-arginine, 1 mM GSH, 1 mM GSSG. The
135 diluted protein was stirred moderately at 4°C for 24 h, concentrated to 25 mL using a
136 stirred cell with a 10-kDa cut-off membrane (Pellicon XL device, Millipore) at 4°C
137 and then filtered using a 0.22-µm syringe filter.

138 **Enzymatic conversion of haem to haemozoin in presence of antimalarial**

139 **compounds**

140 The stock of 10 mM haem was prepared in 0.01 N NaOH. In a buffered reaction with
141 500 mM sodium acetate pH 5.2, 0.5 μM of HDP was added to the reaction and
142 incubated at 37 ºC for 3 h with a final concentration of 600 μM haem. The HDP
143 treated with antimalarial compounds 1 and 2 (50 μM) at 37ºC for 10 min was added to
144 the reaction for assessment of effect of compounds on HDP in an enzymatic
145 conversion of haem to haemozoin. The haem not sequestered into haemozoin was
146 washed thoroughly with 2.5% SDS and 0.1 M sodium bicarbonate (pH 9.1) followed
147 by a distilled water wash until soluble haem was not seen in the supernatant. The
148 haemozoin pellet formed was resuspended in 1 mL of 0.1 N NaOH and the
149 absorbance measured at 400 nm. A reaction with haem alone was used a negative
150 control. The percentage of haemozoin formation was calculated using a molar
151 extinction coefficient of $1x10^5 M^{-1}cm^1$ (34).

152

153 ***In vitro* haemozoin Formation Assay from haemoglobin**

154 Recombinant falcipain 2 and HDP added at a final concentration of 0.5 μM each were
155 mixed with a freshly prepared solution of haemoglobin from red blood cells in a
156 reaction that was buffered with 500 mM sodium acetate pH 5.2, 5 mM reduced
157 glutathione and was incubated at 37ºC for 3 h. Unsequestered haem was repeatedly
158 washed with 2.5% SDS and 0.1 M sodium bicarbonate (pH 9.1) followed by distilled
159 water wash until no soluble haem was visible in the supernatant. The haemozoin
160 pellet was resuspended in 1 mL of 0.1 N NaOH and absorbance was measured at 400
161 nm. A reaction with buffered haemoglobin alone was used as a negative control. The
162 percentage of haemozoin formation was calculated as described above. The reaction

163 with the antimalarial compounds was set with initial addition of falcipain 2 that
164 allowed digestion of haemoglobin. Compounds **1**, 2 (50 μM) or chloroquine (2 μM)
165 were then added to see whether their binding released haem. Subsequently, HDP was
166 added to this reaction to allow haemozoin formation. The amount of haemozoin
167 produced was then calculated as described above.

168 **Supplementary Tables**

169 **TABLE S1** Primers for PCR amplification

Primer ID	**Type**	**Primary PCR amplification**	**Sequence (5'to 3')**	**Codons sequenced**
P1-1 for_P	Primary	*mdr1*, 1st fragment	TAAATGTTTACCTGCACAACATAGAAAAT	
P1-1 rev_P	Primary	*mdr1*, 1st fragment	CTCCACAATAACTTGCAACAGTTCTTA	
P3-1 for_P	Primary	*mdr1*, 2nd fragment	AATTTGATAGAAAAAGCTATTGATTATAA	
P3-1 rev_P	Primary	*mdr1*, 2nd fragment	TATTTGGTAATGATTCGATAAATTCATC	
P5-1 for_P	Primary	*dhfr*	TTTATGATGGAACAAGTCTGC	
P5-1 rev_P SL	Primary	*dhfr*	AGTATATACATCGCTAACAGA	
P8-1 for_P SL	Primary	*dhps*	ATTTTTGTTGAACCTAAACGTGCTGTTCA	
P8-1 rev_P	Primary	*dhps*	CTTGTCTTTCCTCATGTAATTCATCT	
P10-1 for_P	Primary	*crt*, 1st fragment	TTGTCGACCTTAACAGATGGCTCAC	
P10-1 rev_P	Primary	*crt*, 1st fragment	AATTTCCCTTTTTATTTCCAAATAAGGA	
P11-1 for_P	Primary	*crt*, 3rd fragment	ATTTACTCCTTTTTAGATATCACTTA	
P11-1 rev_P	Primary	*crt*, 3rd fragment	TTATATTTTTTAAAAACTATTTCCCTTG	
P12-1 for_P	Primary	*crt*, fifth fragment	AGGAAATAAATATGGGAATGTTTAATTGA	
P12-1 rev_P	Primary	*crt*, fifth fragment	TTCTAAGATAATATTTCCTACACGGT	
P16-1 for_P SS	Primary	*crt*, 4th fragment	TCTGTTATTTTTATTTCTTATAGGCTAT	
P16-1 rev_P SS	Primary	*crt*, 4th fragment	CTTGTATGTATCAACGTTTTTCATCC	
P18-1 for_P SL	Primary	*crt*, 2nd fragment	ACTTTATTTGTATGATTATGTTC	
P18-1 rev_P	Primary	*crt*, 2nd fragment	TAACTGCTCCGAGATAATTGT	
Cytb1_for_P	Primary	*cytb*	CTCTATTAATTTAGTTAAAGCACA	
Cytb2_rev_P	Primary	*cytb*	ACAGAATAATCTCTAGCACC	
P1 for_N	Nested	*mdr1*, 1st fragment	TGTATGTGCTGTATTATCAGGA	61 – 236
P1 rev_N	Nested	*mdr1*, 1st fragment	CTCTTCTATAATGGACATGGTA	
P3 for_N	Nested	*mdr1*, 2nd fragment	GAATTATTGTAAATGCAGCTTTATG	1023 – 1289
P3 rev_N	Nested	*mdr1*, 2nd fragment	GCAGCAAACTTACTAACACG	
P5 for_N	Nested	*dhfr*	ACAAGTCTGCGACGTTTTCGATATTTATG	3 – 216
P5 rev_N SL	Nested	*dhfr*	TAGTATATACATCGCTAACAGAAAT	
P8 for_N	Nested	*dhps*	TTGAAATGATAAATGAAGGTGCTAGT	419 – 647
P8 rev_N SL	Nested	*dhps*	CCAATTGTGTGATTTGTCCAC	
P10 for_N	Nested	*crt,* 1st fragment	CTTGTCTTGGTAAATGTGCTC	39 – 108
P10 rev_N SS	Nested	*crt* 1st fragment	CAATAAAGAACATAATCATACAAATAAAGT	
P11 for_N	Nested	*crt,* 3rd fragment	ACAATTATCTCGGAGCAGTTA	182 - 229
P11 rev_N	Nested	*crt*, 3rd fragment	CATGTTTGAAAAGCATACAGGC	
P12 for_N	Nested	*crt*, fifth fragment	ACCATGACATATACTATTGTTAG	342 – 390
P12 rev_N	Nested	*crt*, fifth fragment	TTATAGAACCAAATAGGTAGCC	
P16 for_N	Nested	*crt*, 4th fragment	CTTTTTCCAATTGTTCACTTCTTG	250 - 335
P16 rev_N SL	Nested	*crt*, 4th fragment	TCTTACATAGCTGGTTATTAAAT	
P18 for_N	Nested	*crt*, 2nd fragment	TCCTTATTTGGAAATAAAAAGGGAAATT	80 - 181
P18 rev_N	Nested	*crt*, 2nd fragment	TAAGTGATATCTAAAAAGGAGTAAAT	
Cytb6_for_N	Nested	*cytb*	CTGCTTTCGTTGGTTATGTCTT	121 - 302

Cytb7_rev_N	Nested	*cytb*	AGTTGTTAAACTTCTTTGTTCTGCT	

170 **TABLE S2** Sensitivity of the selected panel of standard laboratory strains to 72 hours of exposure to various antimalarial compounds

Strain	**Artesunate**		**Chloroquine**		**Mefloquine**		**Pyrimethamine**		**Atovaquone**		**Cycloguanil**	
	IC_{50}[a]	SD	IC_{50}[a]	SD	IC_{50}[a]	SD	IC_{50}[a]	SD	IC_{50}[a]	SD	IC_{50}[a]	SD
NF54	4.5	0.0	7.4	0.9	15.5	0.5	19.5	0.5	0.5	0.0	4.5	0.2
D6	4.1	0.1	8.4	0.2	7.8	1.5	5.5	0.1	0.2	0.1	1.7	0.1
HB3	3.1	0.5	8.4	0.7	8.0	3.1	1082.0	61.0	0.3	0.1	18.5	2.0
FCB	3.8	0.3	83.5	2.0	13.5	1.0	19.0	0.0	0.7	0.1	1164.5	4.0
7G8	1.8	0.2	61.5	2.0	2.5	0.4	>10000	n/a	0.3	0.0	453.0	0.0
K1	2.4	0.5	199.5	17.0	4.1	0.1	9812.0	158.0	0.6	0.0	409.0	9.0
Dd2	5.0	0.5	168.5	19.0	10.9	2.2	9109.0	777.0	0.6	0.2	1965.0	44.0
V1/S	3.5	0.2	305.0	9.0	6.9	0.3	>10000	n/a	0.9	0.1	9693.0	291.0
TM90C2B	3.4	0.4	138.5	25.0	16.0	1.0	>10000	n/a	6195	154.0	6062.5	129.0

171 [a]average median growth inhibition values in nM (n≥2)

172 **TABLE S3** Sensitivity of the selected panel of standard laboratory strains to 72 hours of exposure to compounds 1, 2, and 3 of the 1,2,4-

173 oxadiazole series

Strain	Compound 1		Compound 2		Compound 3	
	IC_{50}[a]	SD	IC_{50}[a]	SD	IC_{50}[a]	SD
NF54	40.5	9.5	58.0	15.0	292.5	52.5
D6	174.0	2.0	274.3	82.1	1045.7	157.8
HB3	57.0	11.0	80.0	14.0	220.0	24.0
FCB	3032.5	14.5	2978.0	227.0	>10000	n/a
7G8	1082.0	180.0	1087.0	160.0	2051.5	359.5
K1	3004.0	333.0	2481.0	363.0	11043.5	1313.5
Dd2	1202.0	358.0	1344.5	66.5	2990.5	586.5
V1/S	2398.5	166.5	2123.5	175.5	6867.0	615.0
TM90C2B	2432.0	139.5	2686.0	272.0	5542.5	12.5

174 [a]average median growth inhibition values in nM (n≥2)

175 **TABLE S4** *In vitro* abiotic β-haematin formation inhibition by compounds **1**, **2**, and **3** of the

176 1,2,4-oxadiazole series

Compound	β-haematin Inhibition IC_{50} (μM)
1	452 ± 65.4
2	587 ± 129
3	*Inactive at 1000 μM*
Amodiaquine	43.5 ± 5.32

177

Figure S1

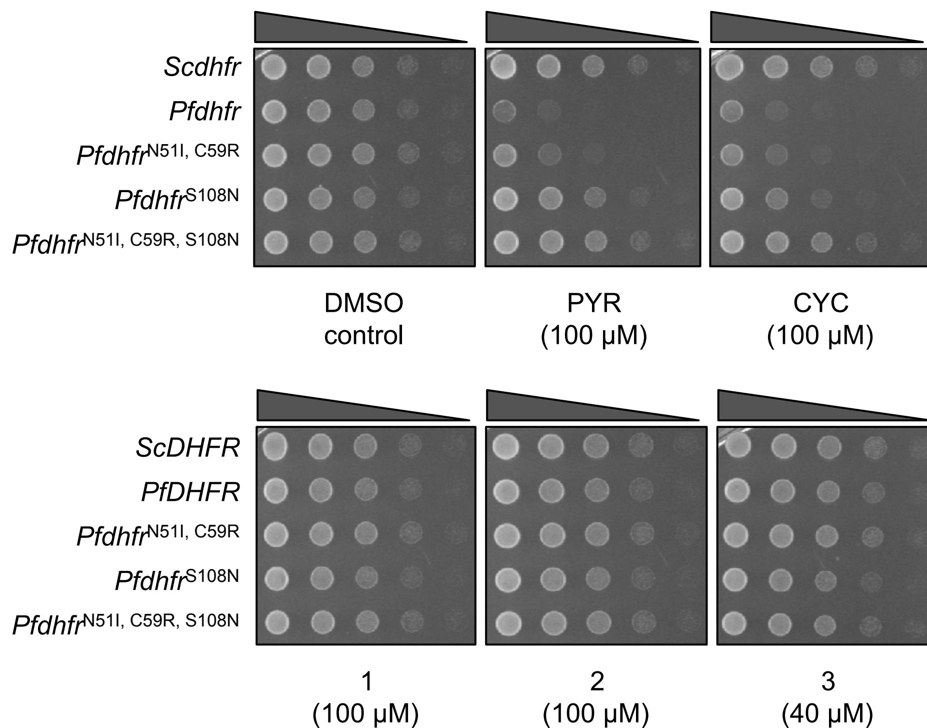


Figure S1 Sensitivity of yeast strains expressing heterologous *dihydrofolate reductase* genes
Serial dilutions (5x) of yeast cultures expressing *Saccharomyces cerevisiae* (*Sc*) and wild-type or mutated *Plasmodium falciparum* (*Pf*) *dhfr* were spotted on agar plates containing a control concentration of DMSO or the indicated concentrations of pyrimethamine (PYR), cycloguanil (CYC), or of the 1,2,4-oxadiazole series compounds (**1**,**2**,**3**). Pyrimethamine inhibits the growth of yeast strains expressing *PfDHFR* as opposed to the native enzyme. Growth inhibition is specifically decreased by *Pfdhfr* mutations N51I, C59R, mutation S108N, and a combination thereof. The 1,2,4-oxadiazole series compounds do not inhibit the growth of any of the yeast strains tested. The highest concentration tested for each compound is reported.

Figure S2

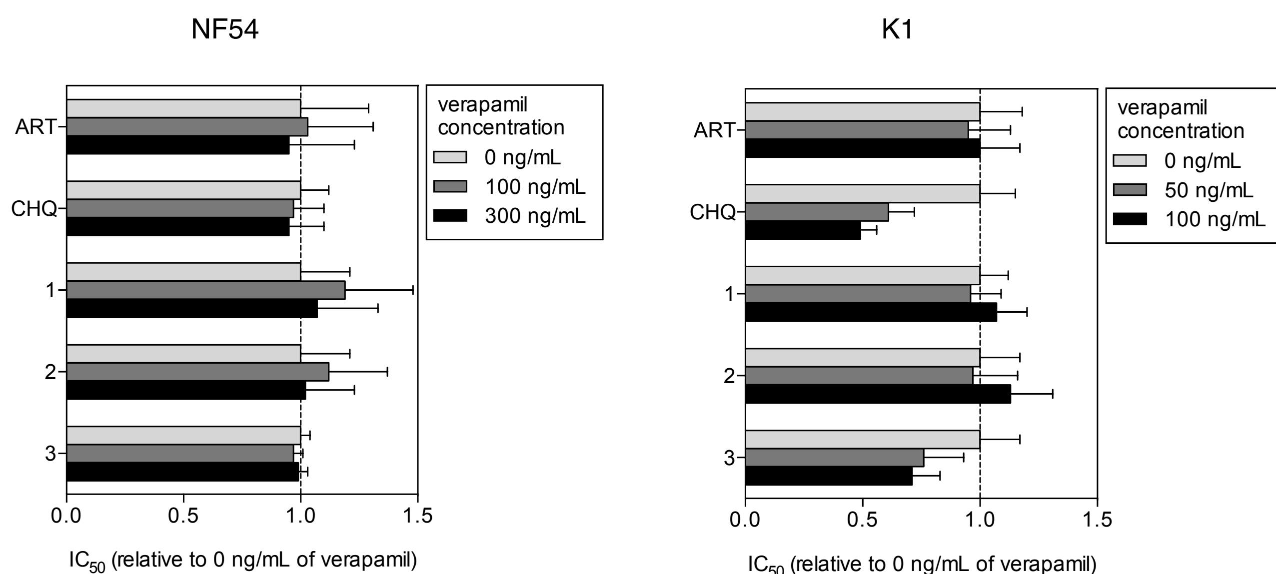
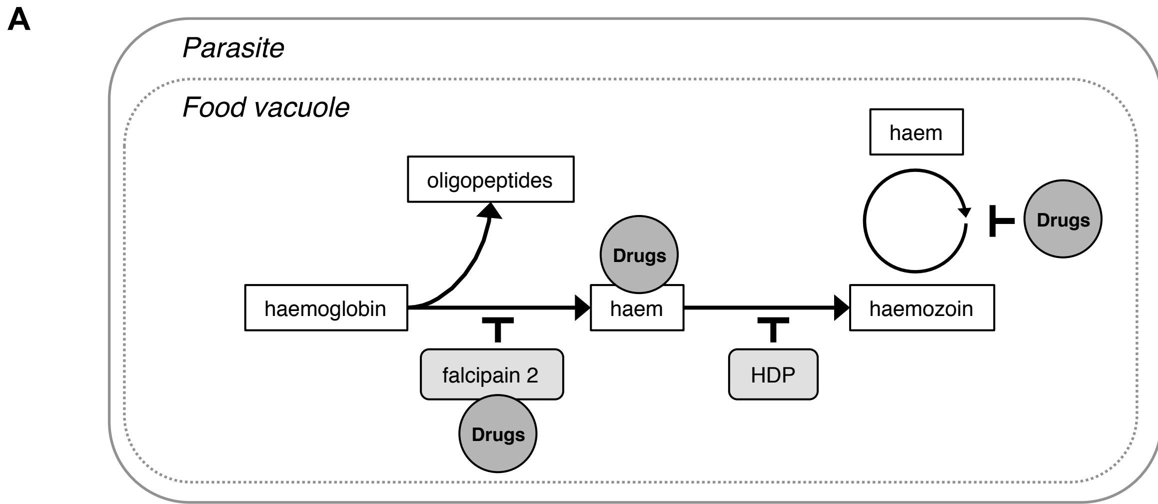


Figure S2 Interaction between verapamil and the 1,2,4-oxadiazole series
The IC_{50} of artesunate (ART), chloroquine (CHQ), and of the 1,2,4-oxadiazole series compounds (**1**,**2**,**3**) in combination with various concentrations of verapamil are indicated for the chloroquino-sensitive strain NF54 and the chloroquino-resistant strain K1. IC_{50} are expressed relative to the 0 ng/mL verapamil condition (n=4, error bars represent SEM). Verapamil induces a specific and dose-dependent increase of K1 sensitivity to chloroquine (2.0 fold increase at 100 ng/mL). A similar effect is apparent with compound **3** only but to a lower extent (1.4 fold increase at 100 ng/mL). Concentrations of 50, 100, and 300 ng/mL of verapamil correspond to 110.1, 220.3, and 660.8 nM, respectively.

Figure S3

A



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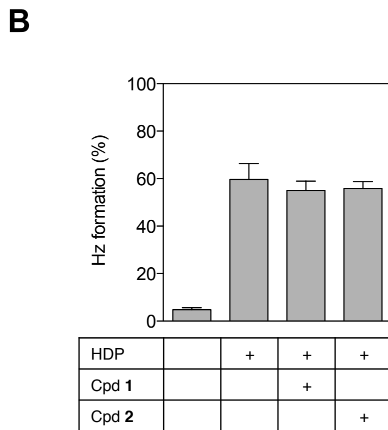
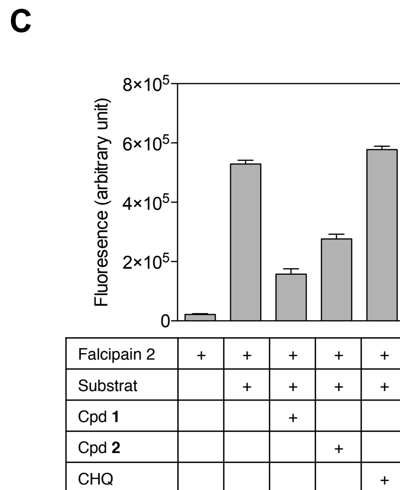


C



D

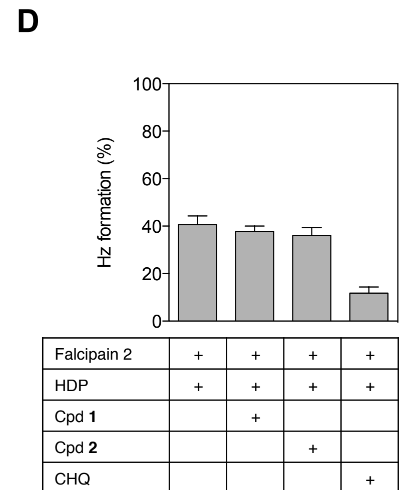


Figure S3. Activity of the 1,2,4-oxadiazole series in haemozoin formation.
(A) Schematic model of the multienzyme haemozoin formation complex in the *Plasmodium falciparum* food vacuole with the potential inhibitory mode of action of drugs. (**B**) Effect of pre-incubation of HDP with 50μM of compounds **1** and **2** on haemozoin formation. (**C**) Effect of compounds **1**, **2**, and chloroquine (CHQ) at 5 μM on falcipain 2 catalytic activity. (**D**) Effect of pre-incubation of falcipain 2 with compound **1**, **2** (50μM), or CHQ (2 μM) on haemozoin formation. For panels B to D, n=2 and error bars represent SD.