Electronic Supplementary Material (ESI) for Medicinal Chemistry Communications

N-substituted Salicylamides as Selective Malaria Parasite Dihydroorotate Dehydrogenase Inhibitors

Ingela Fritzson,*[ab] Paul T. P. Bedingfield,[c] Anders P. Sundin,[b] Glenn McConkey[c] and Ulf J. Nilsson[b]

[a]*Active Biotech AB, PO Box 724, 220 07 Lund, Sweden. E-mail: ingela.fritzson@activebiotech.com; Tel: +46 46 191233*

[b]*Organic Chemistry, Lund University, PO Box 124, 221 00 Lund, Sweden*

[c]*Faculty of Biological Sciences, University of Leeds, Leeds, England LS2 9JT, UK*

Supplementary Information

Chemistry

General methods: Unless otherwise noted, starting materials and reagents were obtained from commercial suppliers. NMR spectra were recorded on a Bruker 500 MHz spectrometer. Chemical shifts, determined from residual solvent peaks, are reported in parts per million relative to $(CH_3)_4Si$ (Table S1). HPLC analysis was performed on a BEH Phenyl, 1.7 µm C18 column (2.1x50 mm) at 50ºC using Acquity UPLC system (Waters) and Acquity PDA detector (Waters) with UV detection at 254 nm (Table S1). The mobile phase consisted of 0.2% HCOOH (solvent A) and acetonitrile (solvent B). The gradient began with 35% solvent B and was linearly increased to 90% for 4 minutes, then the system was returned to initial conditions and allowed to equilibrate for 1 minute before next injection. LC-MS using LTQ XL ion trap with an ESI interface (Thermo Scientific) connected to an Accela UPLC system (Thermo Scientific) confirmed nominal molecular weight (M+H^+) of most intense peak for all synthesized compounds (Table S1). High resolution fast atom bombardment mass spectrometry (FAB-HRMS) was recorded using a JEOL SX-120 instrument (Table S2).

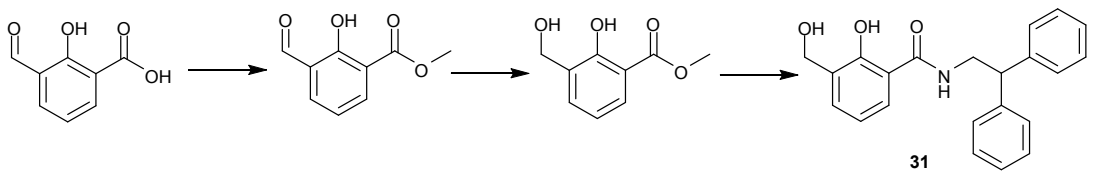
Method A. Procedure for the preparation of compounds **1-13**, **15-16** and **18-30**: The salicylic acid methyl ester (1.0 mmol) was heated with the primary amine (1.0 mmol) in a tube at 110

ºC for 20 hours. To the hot reaction mixture was added 700 µl of toluene and the solution was allowed to cool to room temperature and stand overnight. In case of crystallization, the solid product was filtered off. Otherwise, the toluene solution was chromatographed on silica gel (5 g, Isolute SPE column) using a gradient of heptane/EtOAc (8:1 to 2:1) as eluent. Prepared compounds that did not fulfill the purity criteria (>95%) were stirred in hot ethanol or ethanol/water (9:1), cooled to room temperature and the crystallized salicylamide was isolated by filtration.

When the salicylic acid ester was not available, the corresponding acid was esterified as follows: Methanol (50 ml) was cooled to 0-5 ºC and $SOCl_2$ (1300 µl, 18 mmol) was slowly added in small portions. Thereafter the salicylic acid (4.5 mmol) was added and the reaction mixture was heated at 63 ºC for 4-24 hours until most of the acid had reacted according to TLC (heptane/ EtOAc 2:1). The solvent was evaporated and the residue was dissolved in CH_2Cl_2. The organic solution was washed with 2M $NaHCO_3$ (aq), dried (Na_2SO_4), and evaporated to give the pure salicylic acid methyl ester as a solid.

Method B. Procedure for the preparation of compound **14** and **17**: The salicylic acid (1.0 mmol), primary or secondary amine (1.2 mmol), dicyclohexyl carbodiimide (217 mg, 1.05 mmol) and toluene (3 ml) was stirred and heated to 80 ºC for 4 hours. Thereafter the reaction mixture was allowed to cool to about 40 ºC and formed *N*,*N*'-dicyclohexylurea was filtered off. The toluene solution was chromatographed on silica gel (5 g, Isolute SPE column) using a gradient of heptane/EtOAc (8:1 to 2:1) as eluent to give the pure salicylamide as a solid.

***N*-(2,2-Diphenylethyl)-2-hydroxy-3-(hydroxymethyl)benzamide (31).**

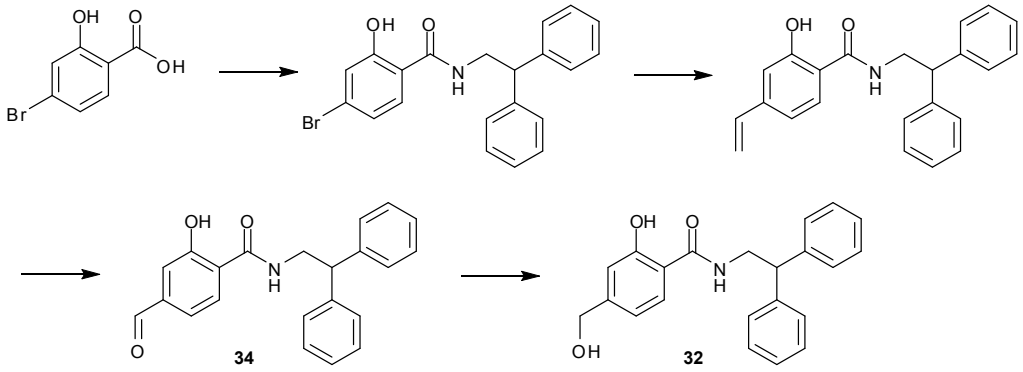


31

Scheme S1.

A solution of 3-formyl-2-hydroxy-benzoic acid (0.56 g, 3.4 mmol), methanol (1.0 ml), dichloroethane (2.0 ml) and H_2SO_4 (0.1 ml) was refluxed for 64 hours. The solvent was removed under vacuum and formed acetal was hydrolyzed by stirring for 1 hour in acetone (3.5 ml) and 2M HCl (0.4 ml). The solvent was removed under vacuum and the residue was dissolved in CH_2Cl_2 (10 ml), washed with 1M $NaHCO_3$ (10 ml), dried (Na_2SO_4), and evaporated to give 3-formyl-2-hydroxy-benzoic acid methyl ester (0.5 g, 82%). The methyl ester (450 mg, 2.5 mmol) dissolved in ethanol (15 ml) was stirred overnight with $NaBH_4$ (113 mg, 3.0 mmol) for reduction of the formyl group. The reaction mixture was concentrated to 3 ml in vacuum, poured into stirred slurry of 2M HCl (10 ml) and ice, and then extracted with $CHCl_3$. The organic phase was dried (Na_2SO_4) and the solvent removed in vacuum to give 2-hydroxy-3-hydroxymethyl-benzoic acid methyl ester (410 mg, 90%). The produced intermediate (220 mg, 1.2 mmol) was refluxed for 10 hours together with 2,2-diphenylethylamine (473 mg, 2.4 mmol) in heptane (50 ml) and condensed heptane was passed through 4Å molecular sieve using a soxhlet extractor to remove formed methanol. The solvent was removed and the residue was chromatographed on silica gel (5 g, Isolute SPE column) using a gradient of heptane/EtOAc (8:1 to 2:1) as eluent to give the solid title compound **31** (20 mg, 4.8%).

***N*-(2,2-Diphenylethyl)-2-hydroxy-4(hydroxymethyl)benzamide (32) and *N*-(2,2-Diphenylethyl)-4-formyl-2-hydroxybenzamide (34).**

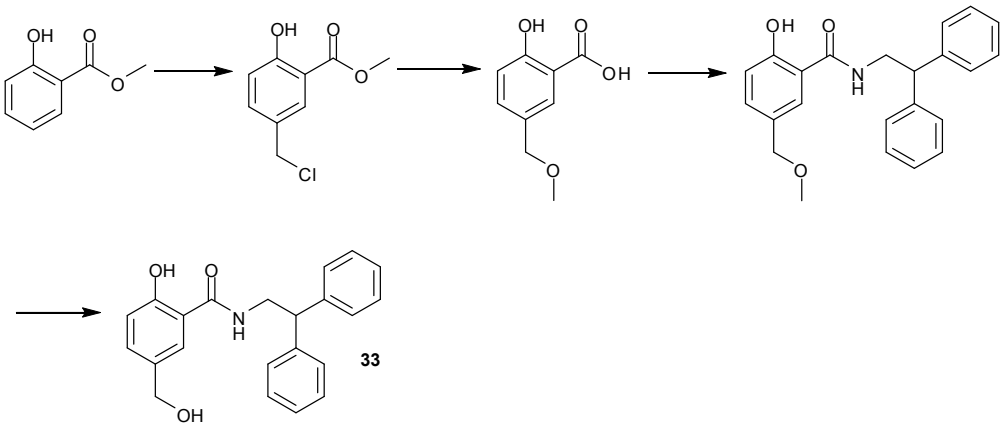


Scheme S2.

A stirred solution of 4-bromo-2-hydroxybenzoic acid (1.1 g, 5 mmol), 2,2-diphenylethylamine (1.1 g, 5.5 mmol) and dicyclohexyl carbodiimide (1.1 g, 5.3 mmol) in toluene (50 ml) was heated to 80 ºC for 4 hours. Thereafter the reaction mixture was allowed to cool to about 40 ºC and formed N,N'-dicyclohexylurea was filtered off. The solution was allowed to stand in room temperature overnight and the crystallized product was filtered off and washed with a small amount of toluene to give *N*-(2,2-diphenylethyl)-2-hydroxy-4-bromobenzamide (1.2 g, 61%). The produced intermediate (713 mg, 1.8 mmol), $NaHCO_3$ (378 mg, 4.5 mmol), $Pd(PPh_3)_4$ (104 mg, 0.09 mmol) and vinyl-boronic acid pinacol ester (416 mg, 2.7 mmol) in dioxane/H_2O (6 ml/1.5 ml)was heated to 95 ºC for 3 hours. Water (20 ml) was added and the mixture extracted with EtOAc (2x20 ml), the organic layer washed with brine, dried (Na_2SO_4), and concentrated to dryness. The residue was recrystallized from toluene to give *N*-(2,2-Diphenylethyl)-4-ethenyl-2-hydroxybenzamide (450 mg, 73%). The produced ethenyl compound (450 mg, 1.3 mmol) was dissolved in $CHCl_3$ and cooled to -78 ºC. A mixture of ozone and oxygen was bubbled through the stirred solution until a blue color persisted. The solution was purged with nitrogen and then triphenylphosphine (2 eq.) on solid support was added and the mixture was allowed to warm to room temperature and stirred for further 4 hours. The solvent was removed and the residue was chromatographed on silica gel (5 g, Isolute SPE column) using a gradient of heptane/EtOAc (8:1 to 4:1) as eluent to give solid title compound **34** (170 mg, 38%). The formyl group was then reduced by dissolving the

benzamide **34** (155 mg, 0.45 mmol) in ethanol (15 ml) and stirring overnight with $NaBH_4$ (21 mg, 0.54 mmol). The reaction mixture was concentrated to 3 ml in vacuum, poured into stirred slurry of 2M HCl (10 ml) and ice, and then extracted with CH_2Cl_2. The organic phase was dried (Na_2SO_4) and the solvent removed in vacuum to give the solid title compound **32** (106 mg, 68%).

***N*-(2,2-Diphenylethyl)-2-hydroxy-5-(hydroxymethyl)benzamide (33).**



Scheme S3.

Salicylic acid methyl ester (15 g, 98 mmol) was stirred in concentrated HCl (37%) and chloromethyl methyl ether (9 g, 254 mmol) was added. After 4 days of stirring a second portion of chloromethyl methyl ether (2 g, 56 mmol) was added and the mixture stirred for an additional 2 days. The crude product was isolated by filtration and dried over solid NaOH in vacuum and then recrystallised twice from petroleum ether (2x100 ml) to yield 5-chloromethyl-2-hydroxy-benzoic acid methyl ester (8.7 g, 44%). The resulting chloro-methylated compound (750 mg, 3.7 mmol) was stirred with 0.5 M NaOMe in MeOH (5 ml) at room temperature over night. The solvent was removed, $CHCl_3$ (20 ml) and water (10 ml) added with stirring to accomplish the hydrolysis of the ester and after a few minutes the solution was acidified with 5 M HCl (0.5 ml). The organic phase was extracted with 1 M $NaHCO_3$ (20 ml) and then acidified with 5M HCl (2 ml). Extraction with $CHCl_3$, drying

(Na_2SO_4), and evaporation yielded 2-hydroxy-5-methoxymethyl-benzoic acid (225 mg, 33%). The benzoic acid (220 mg, 1.2 mmol) was then coupled with 2,2-diphenylethylamine (284 mg, 1.4 mmol) according to general method B to yield *N*-(2,2-Diphenylethyl)-2-hydroxy-5-(methoxymethyl)benzamide (205 mg, 47%). The produced compound was hydrolyzed according to Saá *et al*.[1] Thus, *N*-(2,2-Diphenylethyl)-2-hydroxy-5-(methoxymethyl)benzamide (100 mg, 0.28 mmol) was dissolved in THF (10 ml) and then slowly added to 5M HCl (40 ml) and vigorously stirred for 2 days. The resulting solution was extracted with $CHCl_3$ (2x50 ml) and the combined organic phase was washed with water (50 ml) and dried (Na_2SO_4). The solvent was evaporated and the residue was chromatographed on silica gel (5 g, Isolute SPE column) using a gradient of heptane/EtOAc (4:1 to 1:1) as eluent to give the solid title compound **33** (29 mg, 30%).

Table S1. NMR, HPLC and LC-MS analysis of chemical compounds

Comp. nr	Mol. weight	^{1}H and ^{13}C NMR chemical shifts (ppm)	ESI-MS $[M+H]^+$	HPLC purity
1	241.29	H (DMSO): 12.6 (bs, 1H), 8.9 (bs, 1H), 7.82 (d, J=7.7 Hz, 1H), 7.37-7.41 (m, 1H), 7.28-7.34 (m, 2H), 7.24-7.28 (m, 2H), 7.19-7.24 (m, 1H), 6.85-6.91 (m, 2H), 3.50-3.57 (m, 2H), 2.87 (t, J=7.4 Hz, 2H); C (DMSO): 169.7, 160.9, 140.1, 134.5, 129.5, 129.3, 128.5, 127.1, 119.4, 118.2, 116.1, 41.4, 35.7	242	99.3 %
2	257.29	H (DMSO): 12.6 (bs, 2H), 8.9 (bs, 1H), 7.28-7.32 (m, 2H), 7.23-7.28 (m, 2H), 7.18-7.23 (m, 1H), 7.12 (t, J=8.2 Hz, 1H), 6.32 (d, J=8.2 Hz, 2H), 3.54-3.60 (m, 2H), 2.85 (t, J=7.2 Hz, 2H) C (DMSO): 170.9, 161.2, 140.0, 134.2, 129.5, 129.3, 127.1, 108.0, 103.3, 41.0, 35.6	258	99.5 %
3	275.73	H (DMSO): 13.0 (bs, 1H), 7.51 (dd, J=7.9 Hz, J=1.3 Hz, 1H), 7.35-7.40 (m, 2H), 7.24-7.32 (m, 3H), 7.14 (dd, J=8.0 Hz, J=1.2 Hz, 1H), 6.78 (t, J=7.9 Hz, 1H), 6.4 (bs, 1H), 3.73-3.78 (m, 2H), 2.97 (t, J=6.8 Hz, 2H) C (DMSO): 170.0, 157.9, 140.0, 134.6, 129.5, 129.3, 127.1, 126.7, 122.1, 119.3, 116.6, 41.6, 35.5	276 278	96.2 %
4	275.73	H (DMSO): 12.9 (bs, 1H), 8.9 (bs, 1H), 7.85 (d, J=8.3, 1H), 7.28-7.33 (m, 2H), 7.19-7.27 (m, 3H), 6.95-7.00 (m, 2H), 3.50-3.57 (m, 2H), 2.87 (t, J=7.4 Hz, 2H) C (DMSO):168.7, 161.6, 140.0, 138.4, 130.4, 129.5, 129.3, 127.1, 119.7, 117.9, 115.5, 41.5, 35.6	276 278	99.9 %
5	275.73	H (DMSO): 12.6 (bs, 1H), 9.0 (bs, 1H), 7.91 (d, J=2.6 Hz, 1H), 7.42 (dd, J=8.8 Hz, J=2.7 Hz, 1H), 7.29-7.34 (m, 2H), 7.24-7.28 (m, 2H), 7.19-7.24 (m, 1H), 6.93 (d, J=8.8 Hz, 1H), 3.50-3.56 (m, 2H), 2.86 (t, J=7.4 Hz, 2H); C (DMSO): 168.1, 159.4, 140.0, 134.0, 129.5, 129.3, 128.2, 127.1, 123.1, 120.2, 117.8, 41.5, 35.6	276 278	99.6 %
6	271.31	H (DMSO): 12.8 (bs, 1H), 8.9 (bs, 1H), 7.39 (d, J= 8.1 Hz, 1H), 7.28-7.33 (m, 2H), 7.18-7.27(m, 3H), 7.09 (d, J=7.9 Hz, 1H), 6.81 (t, J=8.0 Hz, 1H), 3.78 (s, 3H), 3.48-3.55 (m, 2H), 2.87 (t, J=7.4 Hz, 2H); C (DMSO): 170.2, 151.7, 149.3, 140.1, 129.5, 129.3, 127.1, 119.4, 118.6, 116.2, 115.8, 56.6, 41.5, 35.7	272	99.5 %

Table S1. NMR, HPLC and LC-MS analysis of chemical compounds

Comp. nr	Mol. weight	^{1}H and ^{13}C NMR chemical shifts (ppm)	ESI-MS $[M+H]^+$	HPLC purity
7	271.31	H (DMSO): 13.1 (bs, 1H), 8.8 (bs, 1H), 7.76 (d, J=8.9 Hz, 1H), 7.28-7.33 (m, 2H), 7.19-7.27 (m, 3H), 6.46 (dd, J=8.9 Hz, J=2.5 Hz, 1H), 6.41 (d, J=2.5 Hz, 1H), 3.77 (s, 3H), 3.47-3.53 (m, 2H), 2.86 (t, J=7.4 Hz, 2H); C (DMSO): 170.0, 164.4, 163.5, 140.2, 129.6, 129.5, 129.2, 127.0, 108.7, 106.8, 102.0, 56.2, 41.3, 35.8	272	98.6 %
8	271.31	H (DMSO): 12.1 (bs, 1H), 8.9 (bs, 1H), 7.39 (d, J=3.1 Hz, 1H), 7.29-7.34 (m, 2H), 7.25-7.28 (m, 2H), 7.20-7.24 (m, 1H), 7.02 (dd, J=9.0 Hz, J=3.1 Hz, 1H), 6.84 (d, J=9.0 Hz, 1H), 3.73 (s, 3H), 3.50-3.56 (m, 2H), 2.87 (t, J=7.4 Hz, 2H); C (DMSO): 169.3, 154.7, 152.3, 140.1, 129.5, 129.3, 127.1, 121.6, 119.0, 116.1, 112.2, 56.5, 41.5, 35.8	272	99.2 %
9	255.32	H (DMSO): 12.2 (bs, 1H), 8.9 (bs, 1H), 7.65 (d, J=1.6 Hz, 1H), 7.28-7.34 (m, 2H), 7.24-7.27 (m, 2H), 7.18-7.24 (m, 2H), 6.79 (d, J=8.3 Hz, 1H), 3.49-3.55 (m, 2H), 2.86 (t, J=7.4 Hz, 2H), 2.24 (s, 3H); C (DMSO): 168.7, 157.7, 139.2, 134.1, 128.5, 128.3, 127.5, 127.0, 126.1, 117.1, 114.8, 40.4, 34.8, 20.0	256	99.4 %
10	286.29	H (DMSO): 13.6 (bs, 1H), 9.3 (bs, 1H), 8.84 (d, J=2.8 Hz, 1H), 8.26 (dd, J=9.1 Hz, J=2.8 Hz, 1H), 7.29-7.34 (m, 2H), 7.25-7.29 (m, 2H), 7.19-7.25 (m, 1H), 7.09 (d, J=9.1 Hz, 1H), 3.54-3.60 (m, 2H), 2.89 (t, J=7.4 Hz, 2H); C (DMSO): 167.5, 166.1, 140.0, 139.9, 129.5, 129.5, 129.3, 127.1, 125.8, 119.3, 116.8, 41.6, 35.5	287	99.7 %
11	227.26	H (DMSO):12.5 (bs, 1H), 9.37 (bs, 1H), 7.91 (dd, J=7.9 Hz, J=1.3 Hz, 1H), 7.39-7.44 (m, 1H), 7.32-7.37 (m, 4H), 7.24-7.29 (m, 1H), 6.88-6.94 (m, 2H), 4.52 (d, J=6.0 Hz, 2H) C (DMSO): 169.8, 160.9, 139.8, 134.7, 129.2, 128.6, 128.1, 127.8, 119.5, 118.3, 116.0, 43.2	228	97.5 %
12	255.32	H (DMSO): 12.7 (bs, 1), 8.86 (bs, 1H), 7.85 (dd, J=7.9 Hz, J=1.3 Hz, 1H), 7.37-7.43 (m, 1H), 7.26-7.32 (m, 2H), 7.22-7.26 (m, 2H), 7.16-7.21 (m, 1H), 6.86-6.92 (m, 2H), 3.29-3.35 (m, 2H), 2.64 (t, J=7.7 Hz, 2H), 1.82-1.90 (m, 2H) C (DMSO): 169.9, 161.1, 142.4, 134.5, 129.2, 128.4, 126.7, 119.3, 118.3, 116.0, 39.5, 33.4, 31.4	256	98.7 %
13	255.32	H (DMSO): 12.5 (bs, 1H), 8.8 (bs, 1H), 7.80 (dd, J=7.9 Hz, J=1.4 Hz, 1H), 7.35-7.40 (m, 1H), 7.26-7.34 (m, 4H), 7.17-7.23 (m, 1H), 6.84-6.89 (m, 2H), 3.41-3.51 (m, 2H), 3.04-3.13 (m, 1H), 1.24 (d, J=7.0 Hz, 3H) C (DMSO): 169.5, 160.7, 145.4, 134.4, 129.3, 128.7, 128.0, 127.2, 119.4, 118.2, 116.3, 46.7, 39.7, 20.2	256	99.8 %
14	303.36	H (DMSO): 12.2 (bs, 1H), 9.49 (d, J=8.4 Hz, 1H), 8.03 (dd, J=7.9 Hz, J=1.2 Hz, 1H), 7.35-7.44 (m, 9H), 7.26-7.33 (m, 2H), 6.89-6.95 (m, 2H), 6.41 (d, J=8.2 Hz, 1H); C (DMSO): 168.3, 160.1, 142.7, 134.6, 129.7, 129.4, 128.3, 128.1, 119.7, 118.1, 116.9, 57.1	304	99.2 %
15	317.39	H (DMSO): 12.3 (bs, 1H), 8.8 (bs, 1H), 7.74 (dd, J=7.9 Hz, J=1.6 Hz, 1H), 7.33-7.37 (m, 5H), 7.28-7.33 (m, 4H), 7.17-7.22 (m, 2H), 6.81-6.87 (m, 2H), 4.42 (t, J=7.9 Hz, 1H), 3.94-3.99 (m, 2H); C (DMSO): 169.2, 160.3, 143.5, 134.4, 129.3, 128.9, 128.8, 127.3, 119.4, 118.1, 116.5, 50.6, 44.3	318	98.9 %
16	331.41	H (DMSO): 12.7 (bs, 1H), 8.9 (bs, 1H), 7.82 (dd, J=7.9 Hz, J=1.4 Hz, 1H), 7.36-7.42 (m, 1H), 7.32-7.36 (m, 4H), 7.27-7.32 (m, 4H), 7.15-7.20 (m, 2H), 6.85-6.91 (m, 2H), 4.05 (t, J=7.8 Hz, 1H), 3.19-3.24 (m, 2H), 2.30-2.37 (m, 2H) C (DMSO): 169.9, 161.1, 145.5, 134.5, 129.3, 128.5, 128.5, 127.0, 119.2, 118.3, 116.0, 49.0, 38.8, 35.0	332	98.0 %
17	255.32	H ($CDCl_3$): 9.8 (bs, 1H), 7.31-7.36 (m, 3H), 7.20-7.29 (m, 4H), 7.01 (d, J=8.3 Hz, 1H), 6.82-6.87 (m, 1H), 3.79 (t, J=7.5 Hz, 2H), 3.11 (s, 3H), 3.01 (t, J=7.5 Hz, 2H); C ($CDCl_3$): 172.1, 159.3, 138.7, 132.9, 129.2, 129.1, 128.6, 127.1, 118.7, 118.3, 117.9, 51.8 (bs), 38.0 (bs), 34.2 (bs)	256	99.7 %
18	351.83	H (DMSO): 13.6 (bs, 1H), 9.2 (bs, 1H), 7.69 (dd, J=8.1 Hz, J=1.4 Hz, 1H), 7.56 (dd, J=7,9 Hz, J=1.3 Hz, 1H), 7.27-7.36 (m, 8H), 7.17-7.22 (m, 2H), 6.85 (t, J=7.9 Hz, 1H), 4.45 (t, J=8.0 Hz, 1H), 3.94-3.99 (m, 2H); C (DMSO): 170.0, 157.5, 143.3, 134.7, 129.3, 128.8, 127.4, 126.7, 122.0, 119.5, 116.6, 50.4, 44.5	352 354	99.9 %

Table S1. NMR, HPLC and LC-MS analysis of chemical compounds

Comp. nr	Mol. weight	^{1}H and ^{13}C NMR chemical shifts (ppm)	ESI-MS $[M+H]^{+}$	HPLC purity
19	351.83	H (DMSO): 12.6 (bs, 1H), 8.81 (bs, 1H), 7.75-7.79 (m, 1H), 7.28-7.37 (m, 8H), 7.16-7.23 (m, 2H), 6.91-6.95 (m, 2H), 4.40 (t, J=7.9 Hz, 1H), 3.94-3.99 (m, 2H); C (DMSO): 168.0, 160.8, 143.4, 138.2, 130.8, 129.4, 128.7, 127.4, 119.7, 117.7, 115.9, 50.6, 44.3	352 354	99.6 %
20	351.83	H (DMSO): 12.4 (bs, 1H), 9.0 (bs, 1H), 7.80 (d, J=2.7 Hz, 1H), 7.28-7.38 (m, 9H), 7.17-7.23 (m, 2H), 6.85 (d, J=8.8 Hz, 1H), 4.38 (t, J=8.0 Hz, 1H), 3.94-3.99 (m, 2H) C (DMSO): 167.6, 158.8, 143.4, 133.9, 129.4, 128.7, 128.5, 127.4, 123.1, 120.1, 118.2, 50.6, 44.4	352 354	99.4 %
21	386.28	H (DMSO): 13.6 (bs, 1H), 9.3 (bs, 1H), 7.85 (d, J=2.4 Hz, 1H), 7.74 (d, J=2.3 Hz, 1H), 7.27-7.37 (m, 8H), 7.18-7.23 (m, 2H), 4.42 (t, J=7.9 Hz, 1H), 3.94-3.99 (m, 2H); C (DMSO): 169.0, 156.5, 143.3, 133.9, 129.4, 128.7, 127.4, 126.3, 123.3, 122.8, 117.2, 50.3, 44.6	386 388 390	99.9 %
22	396.28	H (DMSO): 12.3 (bs, 1H), 8.9 (bs, 1H), 7.94 (d, J=2.5 Hz, 1H), 7.50 (dd, J=8.8 Hz, J=2.5 Hz, 1H), 7.28-7.37 (m, 8H), 7.18-7.23 (m, 2H), 6.84 (d, J=8.8 Hz, 1H), 4.40 (t, J=8.0 Hz, 1H), 3.94-3.99 (m, 2H); C (DMSO): 167.5, 159.2, 143.4, 136.8, 131.4, 129.4, 128.7, 127.4, 120.5, 118.7, 110.6, 50.6, 44.4	396 398	99.0 %
23	335.38	H (DMSO): 12.8 (bs, 1H), 9.0 (bs, 1H), 7.54 (d, J=8.2 Hz, 1H), 7.28-7.38 (m, 9H), 7.17-7.22 (m, 2H), 6.79-6.86 (m, 1H), 4.43 (t, J=7.9 Hz, 1H), 3.94-3.99 (m, 2H); C (DMSO): 169.3 (d, J=3.0 Hz), 152.0 (d, J=242 Hz), 149.4 (d, J=13.3 Hz), 143.4, 129.4, 128.8, 127.4, 123.7 (d, J=3.4 Hz), 120.4 (d, J=17.5 Hz), 118.7 (d, J=5.0 Hz), 118.2 (d, J=3.3 Hz), 50.5, 44.4	336	99.2 %
24	353.37	H (DMSO): 13.3 (bs, 1H), 9.1 (bs, 1H), 7.59-7.63 (m, 1H), 7.28-7.36 (m, 8H), 7.17-7.22 (m, 2H), 6.88-6.96 (m, 1H), 4.42 (t, J=7.9 Hz, 1H) 3.94-3.99 (m, 2H); C (DMSO): 168.8 (d), 154.4 (d), 152.4 (d), 151.6 (dd), 143.3, 141.2 (d), 139.3 (d), 129.4, 128.7, 127.4, 123.9 (dd), 114.3, 107.2 (d), 50.5, 44.4	354	99.0 %
25	335.38	H (DMSO): 11.5 (bs, 1H), 8.3 (bs, 1H), 7.27-7.35 (m, 8H), 7.22-7.27 (m, 1H), 7.17-7.22 (m, 2H), 6.68 (d, J=8.3 Hz, 1H), 6.60-6.64 (m, 1H), 4.39 (t, J=7.9 Hz, 1H), 3.89-3.94 (m, 2H); C (DMSO): 165.5, 161.8, 159.8 (t), 143.5, 132.8 (d), 129.3, 129.8, 127.3, 113.5 (d) 110.2 (d), 106.5 (d), 50.5, 44.4	336	99.9 %
26	331.41	H (DMSO): 12.0 (bs, 1H), 8.8 (bs, 1H), 7.56 (s, 1H), 7.28-7.36 (m, 8H), 7.14-7.23 (m, 3H), 8.75 (d, J=8.3 Hz, 1H), 4.41 (t, J=7.9 Hz, 1H), 3.93-3.98 (m, 2H), 2.20 (s, 3H); C (DMSO): 169.1, 158.1, 143.6, 135.0, 129.4, 128.8, 128.8, 128.0, 127.3, 117.9, 116.1, 50.7, 44.2, 20.9	332	99.9 %
27	347.41	H (DMSO):12.5 (bs, 1H), 8.9 (bs, 1H), 7.26-7.36 (m, 9H), 7.16-7.22 (m, 2H), 7.05 (d, J=7.9 Hz, 1H), 6.75 (t, J=7.8 Hz, 1H), 4.43 (t, J=8.06 Hz, 1H), 3.92-3.97 (m, 2H), 3.75 (s, 3H); C (DMSO):170.0, 151.4, 149.3, 143.5, 129.3, 128.8, 127.3, 119.6, 118.5, 116.1, 116.0, 56.6, 50.5, 44.3	348	95.8 %
28	347.41	H (DMSO): 12.8 (bs, 1H), 8.7 (bs, 1H), 7.66 (d, J=8.9 Hz, 1H), 7.27-7.37 (m, 8H), 7.17-7.23 (m, 2H), 6.42 (dd, J=8.9 Hz, J=2.4 Hz, 1H), 6.37 (d, J=2.4 Hz, 1H), 4.41 (t, J=7.9 Hz, 1H), 3.90-3.95 (m, 2H), 3.74 (s, 3H); C (DMSO): 169.6, 164.3, 162.9, 143.6, 129.9, 129.3, 128.8, 127.3, 109.0, 106.7, 102.0, 56.2, 50.7, 44.2	348	99.6 %
29	347.41	H (DMSO): 11.7 (bs, 1H), 8.8 (bs, 1H), 7.33-7.37 (m, 4H), 7.28-7.33 (m, 5H), 7.18-7.23 (m, 2H), 6.98 (dd, J=8.9 Hz, J=3.1 Hz, 1H), 6.79 (d, J=8.9 Hz, 1H), 4.40 (t, J=7.9 Hz, 1H), 3.94-3.99 (m, 2H), 3.69 (s, 3H); C (DMSO): 168.6, 154.0, 152.3, 143.5, 129.4, 128.8, 127.3, 121.1, 118.9, 116.7, 112.8, 56.5, 50.7, 44.3	348	99.0 %
30	347.41	H (DMSO): 13.7 (s, 1H), 8.5 (bs, 1H), 7.38-7.42 (m, 4H), 7.32-7.38 (m, 4H), 7.29 (t, J=8.3 Hz, 1H), 7.22-7.26 (m, 2H), 4.38 (t, J=8.3 Hz, 1H), 4.00-4.05 (m, 2H), 3.58 (s, 3H); C (DMSO): 169.9, 163.9, 159.2, 143.2, 134.4, 129.5, 128.8, 127.5, 111.5, 104.4, 102.6, 56.9, 50.5, 43.8	348	100.0 %

Table S1. NMR, HPLC and LC-MS analysis of chemical compounds

Comp. nr	Mol. weight	^{1}H and ^{13}C NMR chemical shifts (ppm)	ESI-MS $[M+H]^+$	HPLC purity
31	347.41	H ($CDCl_3$): 12.78 (s, 1H), 7.34-7.40 (m, 5H), 7.26-7.33 (m, 6H), 7.01 (dd, J=8.0 Hz, J=1.3 Hz, 1H), 6.75 (t, J=7.7 Hz, 1H), 6.4 (bs, 1H), 4.73 (s, 2H), 4.33 (t, J=7.9 Hz, 1H), 4.08-4.13 (m, 2H), 2.7 (bs, 1H); C ($CDCl_3$): 170.3, 160.1, 141.9, 133.3, 130.3, 129.3, 128.4, 127.5, 125.0, 118.8, 114.3, 62.2, 50.7, 44.2	348	95.6 %
32	347.41	H (DMSO): 12.3 (bs, 1H), 8.8 (bs, 1H), 7.69 (d, J=8.1 Hz, 1H), 7.28-7.36 (m, 8H), 7.18-7.22 (m, 2H), 6.81 (s, 1H), 6.76 (d, J=8.4 Hz, 1H), 5.3 (bs, 1H), 4.39-4.45 (m, 3H), 3.93-3.98 (m, 2H); C (DMSO):169.2, 160.4, 149.7, 143.5, 129.3, 128.8, 128.7, 127.3, 117.2, 115.3, 114.7, 63.1, 50.6, 44.2	348	97.2 %
33	347.41	H ($CDCl_3$): 12.3 (bs, 1H), 7.25-7.39 (m, 11H), 7.08 (d, J=1.9 Hz, 1H), 6.96 (d, J=8.5 Hz, 1H), 6.4 (bs, 1H), 4.52 (s, 2H), 4.34 (t, J=7.9 Hz, 1H), 4.07-4.12 (m, 2H); C ($CDCl_3$): 170.2, 161.5, 141.9, 133.6, 131.4, 129.3, 128.5, 127.5, 124.5, 119.1, 114.5, 65.0, 50.8, 44.3	348	94.2 %
34	345.40	H (DMSO): 12.3 (bs, 1H), 9.95 (s, 1H), 8.9 (bs, 1H), 7.94 (d, J=8.0 Hz, 1H), 7.28-7.40 (m, 10H), 7.21 (t, J=7.2 Hz, 2H), 4.41 (t, J=8.0 Hz, 1H), 3.97-4.03 (m, 2H); C (DMSO): 193.7, 167.5, 159.7, 143.4, 140.3, 130.4, 129.4, 128.8, 127.4, 122.3, 120.0, 118.5, 50.6, 44.4	346	98.6 %

Table S2. FAB-HRMS for key compounds.

		$[M+H]^+$	
Compound number	Molecular formula	calculated	found
20	$C_{21}H_{18}ClNO_2$	352.1099	352.1117
21	$C_{21}H_{17}Cl_2NO_2$	386.0709	386.0709
24	$C_{21}H_{17}F_2NO_2$	354.1300	354.1310
33	$C_{22}H_{21}NO_3$	348.1594	348.1604

DHODH inhibition assays

For determination of inhibition and IC_{50} values of human and *Plasmodium* DHODH the recombinant DHODH enzymes were used in an *in vitro* enzyme assay with *N*-terminally truncated recombinant DHODH (human[2] and *Plasmodium*[3]). The assay is based on coupling of the ubiquinone reduction to the redox dye 2,6-dichloroindophenol (DCIP).[4] The reduction of DCIP was monitored photometrically by decreasing absorption at 600 nm. The test solutions contained 60 µM DCIP, 150 mM KCl, 50 mM TRIS/HCl pH 7.8, 0.1% Triton X-100, 20 µM decylubiquinone and 200 µM DHO. Synthesized compounds were dissolved in

DMSO and normally added to a final concentration of 1% DMSO. A higher concentration of DMSO, 5%, was used to prevent precipitation and measure inhibition of compounds with IC_{50}'s higher than 100 μM. No inhibition of enzyme function was found in the control containing 5% DMSO.

Parasite growth inhibition assay

P. falciparum (3D7 strain) were cultured in O+ erythrocytes, purified and rinsed in RPMI. The media used was filter sterilised RPMI 1640 with L-Glutamine and 25mM HEPES (Gibco), 5% AlbuMAX (Gibco) 1, 0.2% sodium bicarbonate, 1% human serum in an atmosphere of 1% Oxygen, 3% CO_2, 96% nitrogen. The EC50 assays were conducted in 96 well plates with 200 μL of media per well. The hematocrit was adjusted to 2.5% with 0.5% of the blood cells containing synchronised early trophozoite-stage parasites. Eight concentrations of inhibitors between 500 and 0.01μM were incubated in triplicate with the parasite cultures for 48 hours. 1μCi of [3H] hypoxanthine was added per well and incubated further for 18 hours. Cells were lysed and macromolecules harvested onto glass fiber filters. Experiments were performed in duplicate and performed at least twice. The incorporation of [3H] hypoxanthine (Perkin Elmer) was determined by scintillation counting. Data were plotted as a sigmoidial dose-response curves on GraphPad PRISM to ascertain the 50% inhibitory concentration (EC50).

Molecular modeling

The Schrodinger 2010 software suite (Schrödinger, LLC, New York, NY, 2009) was used for molecular modeling. Energy minimizations were performed with Macromodel, utilizing the

OPLS-2005 force field and the GB/SA model for water solvation, and the protein complexes had the backbone atoms constrained with a force constant of 200 kJ/mol Å^2. Conformational search was performed with the Monte Carlo multiple minima method. The Protein Preparation Wizard was utilized on the crystal structure, to add hydrogens, assign charges, and optimize hydrogen bond networks. The crystal waters with hydrogen bond interactions to the FMN prosthetic group were included in the model.

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