Electronic Supplementary Material (ESI) for Medicinal Chemistry Communications

Supporting information

for

PRIMACINS, *N*-cinnamoyl-primaquine conjugates with improved liver-stage antimalarial activity

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CONTENTS:

1. Detailed synthetic procedures and analytical/spectral data for compounds 5

Synthesis Procedure: in a round bottom flask, the relevant cinnamic acid (1.1 eq., 0.6 mmol) was solubilized in DMF (2.5 mL) and the solution put in an ice bath; then, TBTU (1.1 eq., 0.6 mmol) and DIEA (2 eq., 1.0 mmol) were added and the mixture stirred at 0 ºC for 10 minutes; a solution of primaquine biphosphate in DMF (2.5mL) was then added to the mixture, which was left under stirring for one day at room temperature. Following 50 mL of DCM were added to the reaction mixture and the organic layer was washed with 5% aqueous Na_2CO_3 (3×50mL). Finally, the organic layer was dried over anhydrous Na_2SO_4, filtered, and evaporated under reduced pressure. The residue was submitted to column liquid chromatography on silica, using dichloromethane:acetone (6:1 v/v) as eluant. All target compounds were isolated in high purity degrees and presented correct analytical and spectral data, as given below (Note: NMR peaks assigned to carbons from the heteroaromatic quinoline moiety are assigned as Qn, whereas aromatic carbons from the cinnamoyl moiety are assigned as Ar; others are self-explanatory).

***N*-cinnamoylprimaquine (5a).** Yellow oil (0.13 g, 58%); R_F (DCM/Me_2CO 6:1) 0.49; δ_H (400 MHz, DMSO-d_6) 8.53 (dd, J=4, 1.6Hz, 1H), 7.91 (dd, J=8.4, 1.6Hz, 1H), 7.58 (d, J=15.6Hz, 1H), 7.44 (m, 2H), 7.31 (m, 4H), 6.31 (m, 3H), 5.59 (m, 2H), 3.87 (s, 3H), 3.63 (m, 1H), 3.39 (m, 2H), 1.71 (s, 4H), 1.28 (d, J=6.3Hz, 3H); δ_C (100 MHz, DMSO-d_6) 165.9 (C=O), 159.4 (Qn), 144.8 (Qn), 144.3 (Qn), 140.7 (*trans* CH=CH), 135.3 (Qn), 134.9 (Qn), 134.8 (Ar), 129.9 (Qn), 129.5 (Ar), 128.7 (Ar), 127.7 (Ar), 121.9 (Qn), 120.8 (*trans* CH=CH), 96.9 (Qn), 91.7 (Qn), 55.1 (OCH_3), 47.8 (CH), 39.7 (CH_2), 34.1 (CH_2), 26.1 (CH_2), 20.1 (CH_3); ESI-IT MS: m/z ($M+H^+$) 390.33 ($C_{24}H_{27}N_3O_2$ requires 389.21); HPLC-DAD: t_r = 16.8 min (% area =99.5%).

***N*-(4-methylphenyl)cinnamoylprimaquine (5b).** Yellow oil (0.08 g, 36%); R_F (DCM/Me_2CO 6:1) 0.49; δ_H (400 MHz, DMSO-d_6) 8.53 (dd, J=4, 1.6Hz, 1H), 7.92 (dd, J=8.4, 1.6Hz, 1H), 7.55 (d, J=15.6Hz, 1H), 7.32 (m, 3H), 7.15 (m, 2H), 6.31 (dd, J=15.2, 2.6Hz, 2H), 6.23 (d, J=15.6Hz, 1H), 6.00 (m, 1H), 5.80 (m, 1H), 3.86 (s, 3H), 3.65 (m,1H), 3.39 (m, 2H), 2.35 (s, 3H), 1.73 (s, 4H), 1.30 (d, J=6.4Hz, 3H); δ_C (100 MHz, DMSO-d_6) 166.1 (C=O), 159.4 (Qn), 144.9 (Qn), 144.3 (Qn), 140.7 (*trans* CH=CH), 135.3 (Qn), 134.9 (Qn), 132.1 (Ar), 129.9 (Qn), 129.4 (Ar), 127.7 (Ar), 121.9 (Qn), 119.7 (*trans* CH=CH), 96.9 (Qn), 91.8 (Qn), 55.2 (OCH_3), 47.9 (CH), 39.7 (CH_2), 34.1 (CH_2), 26.1 (CH_2), 21.4 (CH_3), 20.1 (CH_3); ESI-IT MS: m/z ($M+H^+$) 404.27 ($C_{25}H_{29}N_3O_2$ requires 403.23); HPLC-DAD: t_r = 17.6 min (% area = 99.9%).

***N*-(4-isopropylphenyl)cinnamoylprimaquine (5c).** Yellow oil (0.18 g, 78%); R_F (DCM/Me_2CO 6:1) 0.49; δ_H (400 MHz, DMSO-d_6) 8.53 (dd, J=4.4, 1.6Hz, 1H), 7.92 (dd, J=8, 1.6Hz, 1H), 7.56 (d, J=15.6Hz, 1H), 7.37 (m, 2H), 7.30 (m, 1H), 7.19 (m, 2H), 6.32 (dd, J=15.2, 2.6Hz 1H), 6.25 (d, J=15.6Hz, 1H), 6.00 (m, 1H), 5.80 (m, 1H), 5.83 (m, 1H), 3.86 (s, 3H), 3.65 (m,1H), 3.39 (m, 2H), 2.90 (m, 1H), 1.73 (s, 4H), 1.30 (d, J=6.4Hz, 3H), 1.24 (d, J=6.8Hz, 6H); δ_C (100 MHz, DMSO-d_6) 166.1 (C=O), 159.4 (Qn), 150.7 (Ar), 144.9 (Qn), 144.3 (Qn), 140.7 (*trans* CH=CH), 135.3 (Qn), 134.8 (Qn), 132.5 (Ar), 129.9 (Qn), 127.8 (Ar), 126.8 (Ar), 121.9 (Qn), 119.8 (*trans* CH=CH), 96.9 (Qn), 91.8 (Qn), 55.2 (OCH_3), 47.8 (CH), 39.7 (CH_2), 34.1 (CH_2), 34.0 (CH), 26.3 (CH_2), 23.7 (CH_3), 20.6 (CH_3); ESI-IT MS: m/z ($M+H^+$) 432.33 ($C_{27}H_{33}N_3O_2$ requires 431.26); HPLC-DAD: t_r = 19.0 min (% area = 99.9%).

***N*-(4-methoxyphenyl)cinnamoylprimaquine (5d).** Yellow oil (0.19 g, 79%); R_F (DCM/Me_2CO 6:1) 0.49; δ_H (400 MHz, DMSO-d_6) 8.53 (dd, J=4.2, 2Hz, 1H), 7.92 (dd, J=8.4, 2Hz, 1H), 7.53 (d, J=15.6Hz, 1H), 7.38 (m, 2H), 7.29 (m, 1H), 6.86 (m, 2H), 6.32 (dd, J=15.6, 2.4Hz 2H), 6.15

(d, J=15.2Hz, 1H), 6.00 (m, 1H), 5.78 (m, 1H), 3.86 (s, 3H), 3.81 (s, 3H), 3.64 (m,1H), 3.40 (m, 2H), 1.73 (s, 4H), 1.30 (d, J=6.4Hz, 3H); δ_C (100 MHz, DMSO-d_6) 166.2 (C=O), 160.7 (Qn), 159.4 (Ar), 144.9 (Qn), 144.3 (Qn), 140.3 (*trans* CH=CH), 135.3 (Qn), 134.8 (Qn), 129.9 (Qn), 129.3 (Ar), 127.6 (Ar), 121.9 (Qn), 118.4 (*trans* CH=CH), 114.1 (Ar), 96.9 (Qn), 91.8 (Qn), 55.3 (OCH_3), 55.2 (OCH_3), 47.8 (CH), 39.6 (CH_2), 34.1 (CH_2), 26.3 (CH_2), 20.6 (CH_3); ESI-IT MS: m/z ($M+H^+$) 420.30 ($C_{25}H_{29}N_3O_3$ requires 419.22); HPLC-DAD: t_r = 16.7 min (% area = 99.9%).

***N*-(3-fluorophenyl)cinnamoylprimaquine (5e).** Yellow oil (0.13 g, 60%); R_F (DCM/Me_2CO 6:1) 0.49; δ_H (400 MHz, DMSO-d_6) 8.53 (dd, J=4.4, 1.6Hz, 1H), 7.93 (dd, J=8.4, 1.6Hz, 1H), 7.51 (d, J=15.6Hz, 1H), 7.30 (m, 2H), 7.18 (m, 1H), 7.06 (m, 2H), 6.32 (dd, J=17.2, 2.4Hz, 2H), 6.15 (d, J=15.6Hz, 1H), 6.00 (m, 1H), 5.91 (m, 1H), 3.85 (s, 3H), 3.65 (m,1H), 3.40 (m, 2H), 1.74 (s, 4H), 1.29 (d, J=6.4Hz, 3H); δ_C (100 MHz, DMSO-d_6) 165.4 (C=O), 164.2 (Ar), 161.7 (Qn), 159.4 (Qn), 144.8 (Qn), 144.4 (Ar), 139.4 (*trans* CH=CH), 137.2 (Ar), 135.3 (Qn), 134.9 (Qn), 130.2 (Ar), 129.9 (Qn), 123.9 (Ar), 122.0 (Qn), 116.3 (*trans* CH=CH), 113.7 (Ar), 97.0 (Qn), 91.9 (Qn), 55.2 (OCH_3), 47.8 (CH), 39.8 (CH_2), 34.1 (CH_2), 26.1 (CH_2), 20.7 (CH_3); ESI-IT MS: m/z ($M+H^+$) 408.33 ($C_{24}H_{26}FN_3O_2$ requires 407.20); HPLC-DAD: t_r = 17.2 min (% area = 100%).

***N*-(4-fluorophenyl)cinnamoylprimaquine (5f).** Yellow oil (0.17 g, 77%); R_F (DCM/Me_2CO 6:1) 0.49; δ_H (400 MHz, DMSO-d_6) 8.53 (dd, J=4.2, 2Hz, 1H), 7.92 (dd, J=8, 1.6Hz, 1H), 7.53 (d, J=15.6Hz, 1H), 7.40 (m, 2H), 7.31 (m, 1H), 7.02 (m, 2H), 6.32 (dd, J=16, 2.4Hz 2H), 6.18 (d, J=15.6Hz, 1H), 6.00 (m, 1H), 5.86 (m, 1H), 3.86 (s, 3H), 3.65 (m,1H), 3.40 (m, 2H), 1.73 (s, 4H), 1.29 (d, J=6.4Hz, 3H); δ_C (100 MHz, DMSO-d_6) 165.7 (C=O), 159.4 (Qn), 144.9 (Qn), 144.4 (Qn), 139.5 (*trans* CH=CH), 135.3 (Qn), 134.9 (Qn), 129.9 (Qn), 129.5 (Ar), 129.4 (Ar),

121.9 (Qn), 120.5 (*trans* CH=CH), 115.8 (Ar), 115.7 (Ar), 97.0 (Qn), 91.8 (Qn), 55.2 (OCH_3), 47.8 (CH), 39.7 (CH_2), 34.1 (CH_2), 26.2 (CH_2), 20.6 (CH_3); ESI-IT MS: m/z ($M+H^+$) 408.33 ($C_{24}H_{26}FN_3O_2$ requires 407.20); HPLC-DAD: t_r = 17.1 min (% area = 100%).

***N*-(4-chlorophenyl)cinnamoylprimaquine (5g).** Yellow oil (0.15 g, 59%); R_F (DCM/Me_2CO 6:1) 0.49; δ_H (400 MHz, DMSO-d_6) 8.53 (dd, J=4.2, 1.6Hz, 1H), 7.92 (dd, J=8.4, 1.6Hz, 1H), 7.50 (d, J=15.6Hz, 1H), 7.32 (m, 5H), 6.32 (dd, J=16.2, 2.8Hz 2H), 6.22 (d, J=15.6Hz, 1H), 5.99 (m, 1H), 5.88 (m, 1H), 3.86 (s, 3H), 3.65 (m,1H), 3.40 (m, 2H), 1.73 (s, 4H), 1.29 (d, J=6.4Hz, 3H); δ_C (100 MHz, DMSO-d_6) 165.6 (C=O), 159.4 (Qn), 144.9 (Qn), 144.4 (Qn), 139.4 (*trans* CH=CH), 135.3 (Qn), 135.3 (Ar), 134.8 (Qn), 133.4 (Ar), 129.9 (Qn), 128.9 (Ar), 128.9 (Ar), 121.9 (Qn), 121.3 (*trans* CH=CH), 97.0 (Qn), 91.9 (Qn), 55.2 (OCH_3), 47.8 (CH), 39.7 (CH_2), 34.1 (CH_2), 26.2 (CH_2), 20.7 (CH_3); ESI-IT MS: m/z ($M+H^+$) 424.33 ($C_{24}H_{26}ClN_3O_2$ requires 423.17); HPLC-DAD: t_r = 18.0 min (% area = 100%).

***N*-(4-bromophenyl)cinnamoylprimaquine (5h).** Yellow solid (0.19 g, 73%); mp 110-112^oC, R_F (DCM/Me_2CO 6:1) 0.49; δ_H (400 MHz, DMSO-d_6) 8.53 (dd, J=4.4, 1.6Hz, 1H), 7.92 (dd, J=8.2, 1.2Hz, 1H), 7.47 (m, 3H), 7.29 (m, 3H), 6.32 (dd, J=16.2, 2.8Hz 2H), 6.22 (d, J=15.6Hz, 1H), 6.00 (m, 1H), 5.86 (m, 1H), 3.86 (s, 3H), 3.65 (m,1H), 3.40 (m, 2H), 1.73 (s, 4H), 1.30 (d, J=6.4Hz, 3H); δ_C (100 MHz, DMSO-d_6) 165.5 (C=O), 159.4 (Qn), 144.9 (Qn), 144.4 (Qn), 139.4 (*trans* CH=CH), 135.3 (Qn), 134.8 (Qn), 133.8 (Ar), 131.9 (Ar), 129.9 (Qn), 129.1 (Ar), 123.6 (Ar), 121.9 (Qn), 121.4 (*trans* CH=CH), 97.0 (Qn), 91.9 (Qn), 55.2 (OCH_3), 47.8 (CH), 39.7 (CH_2), 34.1 (CH_2), 26.2 (CH_2), 20.7 (CH_3); ESI-IT MS: m/z ($M+H^+$) 468.37 ($C_{24}H_{26}BrN_3O_2$ requires 467.12); HPLC-DAD: t_r = 18.2 min (% area = 100%).

***N*-(2-nitrophenyl)cinnamoylprimaquine (5i).** Orange oil (0.18 g, 75%); R_F (DCM/Me_2CO 6:1) 0.49; δ_H (400 MHz, DMSO-d_6) 8.51 (dd, J=4.4, 1.6Hz, 1H), 7.97 (dd, J=8, 1.2Hz, 1H), 7.89 (m, 2H), 7.52 (m, 3H), 7.28 (m, 1H), 6.30 (dd, J=10.2, 2.4Hz, 2H), 6.20 (d, J=15.2Hz, 1H), 6.07 (m, 1H), 5.98 (m, 1H), 3.85 (s, 3H), 3.63 (m,1H), 3.40 (m, 2H), 1.72 (s, 4H), 1.29 (d, J=6.4Hz, 3H); δ_C (100 MHz, DMSO-d_6) 164.9 (C=O), 159.4 (Qn), 148.2 (Ar), 144.8 (Qn), 144.3 (Qn), 135.7 (*trans* CH=CH), 135.3 (Qn), 134.8 (Qn), 133.2 (Ar), 131.1 (Ar), 129.9 (Qn), 129.6 (Ar), 129.1 (Ar), 126.2 (Ar), 124.7 (Qn), 121.9 (*trans* CH=CH), 96.9 (Qn), 91.8 (Qn), 55.2 (OCH_3), 47.8 (CH), 39.8 (CH_2), 34.1 (CH_2), 26.1 (CH_2), 20.6 (CH_3); ESI-IT MS: m/z ($M+H^+$) 435.40 ($C_{24}H_{26}N_4O_4$ requires 434.20); HPLC-DAD: t_r = 16.7 min (% area = 100%).

***N*-(3-nitrophenyl)cinnamoylprimaquine (5j).** Orange oil (0.16 g, 65%); R_F (DCM/Me_2CO 6:1) 0.49; δ_H (400 MHz, DMSO-d_6) 8.53 (dd, J=4.4, 1.6Hz, 1H), 8.26 (m, 1H), 8.15 (m, 1H), 7.92 (dd, J=8, 1.6Hz, 1H), 7.68 (d, J=7.6Hz, 1H), 7.58 (d, J=15.6Hz, 1H), 7.50 (m, 1H), 7.30 (m, 1H), 6.33 (m, 3H), 6.01 (m, 2H), 3.84 (s, 3H), 3.65 (m,1H), 3.42 (m, 2H), 1.73 (s, 4H), 1.29 (d, J=6.4Hz, 3H); δ_C (100 MHz, DMSO-d_6) 164.9 (C=O), 159.4 (Qn), 148.5 (Ar), 144.8 (Qn), 144.4 (Qn), 138.0 (*trans* CH=CH), 136.7 (Ar), 135.3 (Qn), 134.9 (Qn), 134.0 (Ar), 129.9 (Qn), 129.8 (Ar), 123.8 (Ar), 123.8 (Ar), 121.9 (Qn), 121.5 (*trans* CH=CH), 97.0 (Qn), 91.9 (Qn), 55.2 (OCH_3), 47.8 (CH), 39.8 (CH_2), 34.1 (CH_2), 26.0 (CH_2), 20.6 (CH_3); ESI-IT MS: m/z ($M+H^+$) 435.27 ($C_{24}H_{26}N_4O_4$ requires 434.20); HPLC-DAD: t_r = 17.1 min (% area = 99.8%).

***N*-(4-nitrophenyl)cinnamoylprimaquine (5k).** Orange oil (0.16 g, 65%); R_F (DCM/Me_2CO 6:1) 0.49; δ_H (400 MHz, DMSO-d_6) 8.52 (dd, J=4.4, 1.6Hz, 1H), 8.16 (d, J=8.8Hz, 2H), 7.92 (dd, J=8.4, 1.6Hz, 1H), 7.55 (m, 3H), 7.30 (m, 1H), 6.34 (m, 3H), 6.01 (m, 1H), 5.98 (m,1H), 3.85 (s, 3H), 3.65 (m,1H), 3.40 (m, 2H), 1.74 (s, 4H), 1.29 (d, J=6.4Hz, 3H); δ_C (100 MHz, DMSO-d_6)

164.8 (C=O), 159.4 (Qn), 148.0 (Ar), 144.8 (Qn), 144.4 (Qn), 141.2 (*trans* CH=CH), 138.0 (Ar), 135.3 (Qn), 134.9 (Qn), 129.9 (Qn), 128.2 (Ar), 125.0 (Ar), 124.0 (Qn), 122.0 (*trans* CH=CH), 97.1 (Qn), 91.9 (Qn), 55.2 (OCH_3), 47.9 (CH), 39.9 (CH_2), 34.2 (CH_2), 26.0 (CH_2), 20.6 (CH_3); ESI-IT MS: m/z ($M+H^+$) 435.26 ($C_{24}H_{26}N_4O_4$ requires 434.20); HPLC-DAD: t_r = 17.1 min (% area = 100%).

2. Procedures for *in vitro Plasmodium* liver stage infection assays

Inhibition of liver stage infection by compounds **5** was determined by measuring the luminescence intensity in Huh-7 cells infected with a firefly luciferase-expressing *P. berghei* line, *Pb*GFP-Luc_{con}, as previously described.[9]

Huh-7 cells, a human hepatoma cell line, were cultured in 1640 RPMI medium supplemented with 10% v/v fetal calf serum, 1% v/v non-essential amino acids, 1% v/v penicillin/streptomycin, 1% v/v glutamine and 10 mM 4-(2-hydroxyethyl)-1-piperazine-ethanesulphonic acid (HEPES), pH 7, and maintained at 37 °C with 5% CO_2.

For infection assays, Huh-7 cells (1.2×10^4 per well) were seeded in 96-well plates the day before drug treatment and infection. Medium in the cells was replaced by medium containing the appropriate concentration of each compound approximately 1 h prior to infection with sporozoites freshly obtained through disruption of salivary glands of infected female *Anopheles stephensi* mosquitoes. Sporozoite addition was followed by centrifugation at 1700*g* for 5 min. At 24 h post-infection, medium was replaced by fresh medium containing the appropriate concentration of each compound. Inhibition of parasite development was measured 48 h after infection.

The effect of the compounds on the viability of Huh-7 cells was assessed by the AlamarBlue assay (Invitrogen, UK), using the manufacturer's protocol.

3. Procedures for *in vitro Plasmodium* blood stage infection assays

These assays were conducted as previously described by us.[7] Briefly, synchronized ring-stage W2 strain *P. falciparum* parasites were cultured with multiple concentrations of test compounds (added from 1,000× stocks in DMSO) in RPMI 1640 medium with 10% human serum. After a 48 h incubation, when control cultures contained new rings, parasites were fixed with 1% formaldehyde in PBS, pH 7.4, for 48 h at room temperature and then labeled with YOYO-1 (1 nM; Molecular Probes) in 0.1% Triton X-100 in PBS. Parasitemias were determined from dot plots (forward scatter vs. fluorescence) acquired on a FACSort flow cytometer using CELLQUEST software (Becton Dickinson). IC_{50}s for growth inhibition were determined with GraphPad Prism software from plots of percentages of the level of parasitemia of the control relative to inhibitor concentration. In each case, goodness of curve fit was documented by R^2 values of > 0.95.