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Reversed Chloroquines Based on the 3,4-Dihydropyrimidin-2(1*H*)-one Scaffold: Synthesis and Evaluation for Antimalarial, β-Haematin Inhibition, and Cytotoxic Activity

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The control of malaria is complicated by the increasing resistance of malaria parasites to effective available drugs, particularly to the cheap and once effective drug, chloroquine (CQ) **1** (Figure 1).[1] Some of the current strategies in malaria control focus on various approaches to revive CQ-based drugs to combat the dilemma of drug resistance. One strategy involves the search for drugs known as reversing agents (RAs), which may be used in combination with CQ.[2]

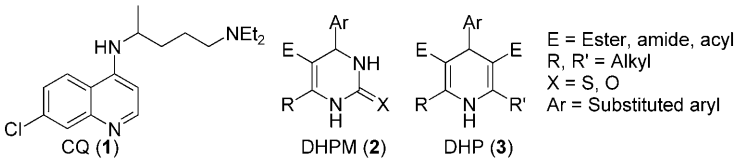

Figure 1. Structure of chloroquine (CQ) **1**, 4-aryl-3,4-dihydropyrimidin-2(1*H*)-ones (DHPM) **2**, and 4-aryl-1,4-dihydropyrimidines (DHP) **3**.

Recently the effectiveness of linking an RA to a CQ-like moiety was demonstrated by Peyton and co-workers.[3] Their work included the design and synthesis of the so-called "reversed chloroquines" (RCQs) in which a CQ-like moiety and an RA were covalently linked to yield highly effective agents against the *P. falciparum* CQ-resistant (CQR) and CQ-sensitive (CQS) parasites in vitro and in vivo. These types of drugs were proposed to exert their activity by targeting two mechanisms. It was suggested that both low digestive vacuole (DV) pH and binding of CQ to haematin, thus preventing haemozoin formation, would enhance accumulation of both drug components within the DV. In addition to enhanced accumulation, it was postulated that the RA component would interfere with efflux from the DV of the CQ moiety by the mutated CQ-resistant PfCRT transport protein. Kyle et al.[4] previously evaluated interactions between various Ca^{2+} channel modulators and quinoline-containing antimalarials and found that verapamil and two analogues, diltiazem and chlorpromazine, act synergistically in vitro with CQ, desethyl-CQ, quinine, and quinidine against the resistant strains. Based on these findings we decided to turn our attention to RCQs based on the 4-aryl-3,4-dihydropyrimidin-2(1*H*)-ones (DHPMs) **2**, which are related to the most widely studied class of organic calcium channel modulators, the 4-aryl-1,4-dihydropyridines (DHPs) **3**[5] (Figure 1). The Biginelli multicomponent reaction used to construct DHPMs would facilitate structure–activity relationship studies.

The first step of the synthesis involved a one-pot reaction between aldehyde **4**, β-keto ester **5**, and urea **6** in the presence of a catalytic amount of $CeCl_3 \cdot 7H_2O$ to afford the Biginelli DHPM **7**[6] (Scheme 1). The mono- and dimethylated DHPMs (**8** and **9**, respectively) were obtained by reaction of **7** with methyl iodide.[7] The monomethylated DHPM **8** was converted into carbamate **10**, which was subsequently transformed into the respective 4-aminoquinoline DHPM derivatives **14**–**16** by reaction with diamines **11**–**13**.[8]

In an attempt to improve the aqueous solubility of target compounds **14**–**16**, selected citrate salts **17**–**19**[9] were also prepared for biological evaluation. The data for the antiplasmodial screening of compounds **14**–**16** and citrate salts **17**–**19** against the CQ-sensitive (CQS) 3D7 and CQ-resistant (CQR) K1 *P. falciparum* strains is shown in Table 1. The IC_{50} range over triplicate determinations are included in brackets. Almost all compounds in the 3D7 assay exhibited IC_{50} values similar to CQ, with the exception of **15** ($IC_{50} = 0.1$ μM), which displayed a notably lower activity.

Against the K1 strain, all compounds were more active than CQ ($IC_{50} = 0.853$ μM), with the most active, **16** ($IC_{50} = 4$ nM), displaying an activity 200-fold greater than CQ. The IC_{50} values of the other five compounds (**14**, **15**, and **17**–**19**) ranged from 0.100 to 0.144 μM, approximately eightfold greater than CQ. With the exception of **14** and **16**, the compounds showed modest toxicity towards the mammalian KB cell line employed in the assay, as demonstrated by their lower therapeutic indices (see Table 1). The most notable compounds are **16** and its corresponding citrate salt **19**, which had the most outstanding activity and therapeutic index against the resistant K1 strain. With the exception of these two compounds, lower activity against K1 than 3D7 was observed in the other cases.

The mechanism of action of CQ is believed to involve inhibition of haemozoin formation, and any compound demon-

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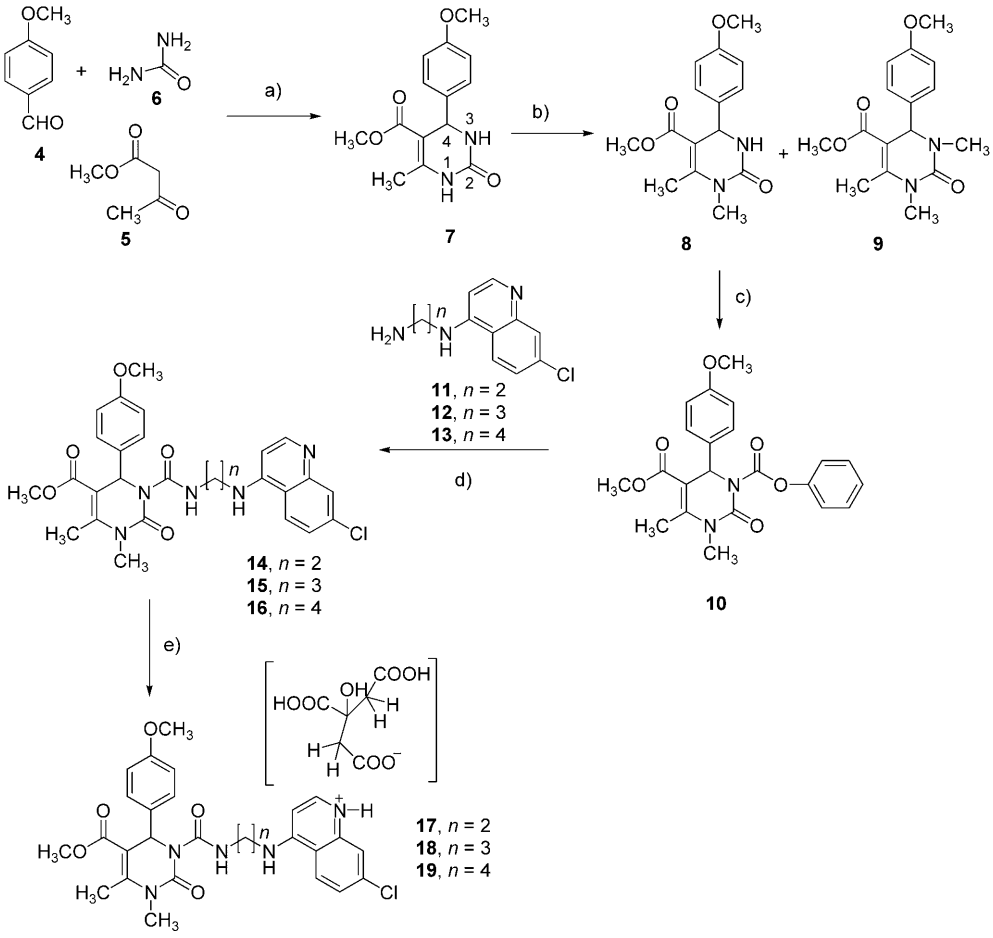

Scheme 1. Reagents and conditions: a) $CeCl_3 \cdot 7H_2O$, EtOH, reflux, 3 h, 94%; b) NaH (1.1 equiv), anhydrous DMF, 0 °C, 30 min; MeI (1.0 equiv), room temperature, 45 min, **8** (76%), **9** (6%); c) NaH (11.0 equiv), 0 °C; phenyl chloroformate (10.0 equiv), THF, 60 °C, 86%; d) K_2CO_3, THF, respective diamines,[8] N_2 atmosphere, 25 °C, 15 h, 70–97%; e) citric acid, acetone, 5 °C, 24 h, 57–95%.

Table 1. Antiplasmodial activities of DHPMs **14**–**16** and citrate salts **17**–**19**.

Compound	*n*	IC_{50} [nM] 3D7 (range)	IC_{50} [nM] K1 (range)	Cyto [μM][d]	TI[e] 3D7	TI[e] K1
CQ[a]	–	14	853 (57–1300)	–	–	–
ART[b]	–	5	1 (0.95–1)	–	–	–
POD[c]	–	–	–	0.004	–	–
14	2	10 (1–16)	100 (60–140)	56.86	5686	569
15	3	100 (65–170)	100 (100–320)	21.66	217	217
16	4	28 (16–51)	4 (2–9)	8.89	318	2223
17	2	32 (20–50)	131 (120–150)	31.40	981	240
18	3	62 (40–80)	144 (130–160)	26.97	435	187
19	4	7 (2–20)	8 (0.2–3)	4.97	355	6212

[a] Chloroquine. [b] Artesunate. [c] Podophyllotoxin. [d] Cytotoxicity. [e] Therapeutic index.

strating such activity represents a possible antimalarial drug candidate. To shed light on a possible mode of action of RCQs **14**–**16**, these compounds and related citrate salts were also tested for their ability to inhibit the formation of β-haematin (synthetic haemozoin) (see Table 2). All the citrate salts **17**–**19** were found to be several times more active than CQ. The IC_{50} values for β-haematin inhibition ranged from 0.248 to 0.870 equivalents, and the most potent compound, **17** (IC_{50} = 0.248 equiv), was almost eight-fold more active than CQ (IC_{50} = 1.91 equiv). Overall, the citrate salts displayed greater inhibitory activity than the free DHPMs, presumably due to their improved solubility under the assay conditions. Surprisingly, the same trend was not observed in the antiplasmodial activity assay, although in most cases the citrate salts and free DHPMs displayed similar activities.

In terms of SAR studies, no direct linear correlation between chain length and antiplasmodial activity was observed for the structurally related compounds **14**–**16**. In most assays the citrate salt derivatives were more potent than their corresponding parent free bases, presumably due to improved solubility. There is a fairly good correlation between the antimalarial activity against the CQR strain and inhibition of β-haematin formation. A correlation is also observed in 3D7 with the exception of **14**. This correlation may be a result of similar pK_a values, and thus similar vacuolar accumulation of the dihydropyrimidinone derivatives is independent of the chloroquine resistance mechanism. Slight deviations in the correlation between the in vitro antimalarial activity and β-haematin inhibition may be a result of the small differences in pK_a values. The correlation, or lack thereof, between biological activity and β-haematin inhibition is normally investigated in the CQS strains because other factors come into play in CQR strains that may prevent a direct comparison.

All the tested compounds exhibited excellent antiplasmodial activities against the K1 resistant strain. These compounds also displayed good activity against β-haematin formation. These results suggest that these DHPM–CQ conjugates may exert bifunctional antimalarial effects by acting as reversal agents as well as inhibitors of β-haematin formation. Drug development

Table 2. IC_{50} values for β-haematin inhibition[a] of DHPMs **14**–**16** and citrate salts **17**–**19**.

Compound	*n*	IC_{50} [equiv][b]
CQ	–	1.91 ± 0.30
DHPM	–	Not active
14	2	0.870 ± 0.08
15	3	0.862 ± 0.03
16	4	0.464 ± 0.02
17	2	0.248 ± 0.02
18	3	0.467 ± 0.02
19	4	0.269 ± 0.01

[a] Experimental protocol outlined in reference [11]. [b] Results are the mean ± SE of three determinations.

strategies that rely on known parasite-specific targets and build on the existing battery of antimalarial compounds are likely to yield an effective antimalarial therapy.

Experimental Section

^{1}H NMR spectra were recorded at ambient temperature using a Varian Mercury (300 MHz) or a Varian Unity Spectrometer (400 MHz), and TMS was used as an internal standard. Chemical shift values (δ) are given in ppm relative to TMS ($\delta = 0.00$). ^{13}C NMR spectra were recorded at 75 or 100 MHz with the same instruments and internal standard. Deuterated methanol (CD_3OD) and chloroform ($CDCl_3$) were used in the determination of spectra for the amines and DHPMs, respectively. Mass spectra were recorded by means of a VG Micromass 16F spectrometer at 70 eV with an accelerating voltage of 4 kV. Accurate masses were determined using a VG-70E spectrometer and VG (Micromass) 70-SE magnetic sector mass spectrometer. IR spectra were measured in solution using chloroform on a satellite FTIR spectrophotometer. Melting points were determined on a Reichert-Jung Thermovar (temperature range 0–350 °C) on cover slips and are uncorrected. Reactions were monitored by TLC using silica gel coated plates, and visualised under UV light.

4-(4-Methoxyphenyl)-6-methyl-2-oxo-1,2,3,4-tetrahydropyrimidine-5-carboxylic acid methyl ester (7): A solution of methyl acetoacetate **4** (1.16 g, 10.00 mmol), 4-methoxybenzaldehyde **5**, (1.36 g, 10 mmol) and urea **6** (1.80 g, 30.00 mmol) in EtOH (15 mL) was heated at reflux in the presence of $CeCl_3{\cdot}7H_2O$ (931 mg, 25.00 mmol%) for 3 h. After complete conversion of starting material to product (monitored by TLC), the reaction mixture was cooled to ambient temperature, poured into crushed ice (30 g) and stirred for 15 min, resulting in the formation of a white precipitate. The precipitate was filtered by suction, washed with ice-cold H_2O (50 mL), and recrystallised from hot EtOH to afford the pure product, **7** as white needles (2.62 g, 94%); mp: 202–204 °C (lit.: 201–203 °C[10]); ^{1}H NMR (400 MHz, CD_3OD): $\delta = 7.21$ (2H, d, $J =$ 8.6 Hz, H-2′ and H-6′), 6.86 (2H, d, $J = 8.6$ Hz, H-3′ and H-5′), 5.25 (1H, s, H-4), 3.79 (3H, s, CO_2Me), 3.75 (3H, s, $ArOCH_3$), 2.32 ppm (3H, s, 6-CH_3); ^{13}C NMR (100 MHz, CD_3OD): $\delta = 166.9$ (C=O), 159.5, 154.0 (C=O), 147.5, 136.7, 127.6 (2C), 113.8 (2C), 101.1, 54.5, 48.7, 50.3, 16.9 ppm.

4-(4-Methoxyphenyl)-1,6-dimethyl-2-oxo-1,2,3,4-tetrahydropyrimidine-5-carboxylic acid (8) and 4-(4-methoxyphenyl)-1,3,6-trimethyl-2-oxo-1,2,3,4-tetrahydropyrimidine-5-carboxylic acid methyl ester (9): A solution of **7** (3.0 g, 10.90 mmol) in *N*,*N*-dimethylformamide (DMF, 5 mL) at 0 °C was added to a suspension of a 60% oil dispersion of NaH (0.478 g (60%), 11.99 mmol) in DMF (10 mL) at 0 °C. After the mixture was stirred for 30 min at 0 °C, a solution of methyl iodide (1.70 g, 11.99 mmol) in DMF (1 mL) was added, and stirring was continued at 25 °C for 45 min. TLC analysis indicated complete conversion of starting material to products. The reaction mixture was diluted with H_2O (50 mL) and extracted with EtOAc (3×50 mL). The organic extracts were combined, washed with brine (50 mL), H_2O (5×50 mL), dried over $MgSO_4$, filtered and concentrated in vacuo to yield a white residue. The desired pure product **8** and a byproduct, **9**, were separated by column chromatography on SiO_2 gel using Et_2O/CH_2Cl_2 (1:9) as eluent.

4-(4-Methoxyphenyl)-1,6-dimethyl-2-oxo-1,2,3,4-tetrahydropyrimidine-5-carboxylic acid (8): White solid (0.40 g, 76%): $R_f = 0.56$ (Et_2O/CH_2Cl_2 1:9); mp: 149–151 °C; ^{1}H NMR (400 MHz, $CDCl_3$): $\delta =$ 7.18 (2H, d, $J = 8.8$ Hz, H-2′ and H-6′), 6.82 (2H, d, $J = 8.8$ Hz, H-3′ and H-5′), 5.60 (1H, s, NH), 5.38 (1H, s, H-4), 3.77 (3H, s, CO_2Me), 3.64 (3H, s, $ArOCH_3$), 3.21 (3H, s, NCH_3-1), and 2.50 (3H, s, CH_3-6); ^{13}C NMR (100 MHz, $CDCl_3$): $\delta = 167.0$ (C=O), 159.0, 154.0 (C=O), 149.5, 135.0, 127.5 (2C), 114.3 (2C), 110.0, 55.5, 53.5, 51.5, 30.5, 16.8 ppm; IR (CH_2Cl_2): $\tilde{\nu} = 3300$ (N–H), 3052 (C–H), 1696 (C=O) and 1628 cm^{-1}(C=O); LRMS (EI): *m/z* (%): 290 (60) $[M+H]^+$; Anal. calcd for $C_{15}H_{18}N_2O_4$: C 62.06, H 6.25, N 9.65%, found: C 62.15, H 6.16, N 9.57%.

4-(4-Methoxyphenyl)-1,3,6-trimethyl-2-oxo-1,2,3,4-tetrahydropyrimidine-5-carboxylic acid methyl ester (9): White solid (0.19 g, 6%); $R_f = 0.18$ (Et_2O/CH_2Cl_2 1:9); mp: 86–87 °C; ^{1}H NMR (400 MHz, $CDCl_3$): $\delta = 7.18$ (2H, d, $J = 8.8$ Hz, H-2′ and H-6′), 6.82 (2H, d, $J =$ 8.8 Hz, H-3′ and H-5′), 5.20 (1H, s, H-4), 3.78 (3H, s, CO_2Me), 3.67 (3H, s, $ArOCH_3$), 3.26 (3H, s, NCH_3-1), 2.90 (3H, s, NCH_3-3), 2.73 ppm (3H, s, CH_3-6); ^{13}C NMR (100 MHz, $CDCl_3$): $\delta = 167.0$ (C=O), 159.0, 154.0 (C=O), 149.5, 135.0, 127.9 (2C), 114.2 (2C), 110.0, 55.5, 53.5, 51.5, 32.0, 30.0, 16.8 ppm; IR (CH_2Cl_2): $\tilde{\nu} = 3398$ (N–H), 3052 (C–H), 1778 (C=O) and 1638 cm^{-1} (C=O); LRMS (EI): *m/z* (%) 304 (75) $[M+H]^+$; Anal. calcd for $C_{16}H_{20}N_2O_4$: C 63.14, H 6.62, N 9.20%, found: C 63.25; H 6.58; N 9.15%.

6-(4-Methoxyphenyl)-3,4-dimethyl-2-oxo-3,6-dihydro-2*H*-pyrimidine-1,5-dicarboxylic acid 5-methyl ester 1-phenyl ester (10): Phenyl chloroformate (10.28 g, 8.293 mL, 65.67 mmol) was added dropwise to a mixture of monomethylated DHPM **8**, (1.90 g, 6.58 mmol) and a 60% oil suspension of NaH (2.89 g (60%), 72.24 mmol) in THF (100 mL) at 0 °C. The mixture was allowed to stir for 12 h at 60 °C. Thereafter, excess NaH was quenched by adding H_2O dropwise until effervescence stopped. The product was then extracted with EtOAc (3×100 mL), washed with H_2O (3× 50 mL), dried over anhydrous $MgSO_4$, and the solvent was evaporated in vacuo. The product was purified on a silica gel column using a mobile phase of 10–50% EtOAc in hexane. White foam (2.30 g, 85%): $R_f = 0.12$ (EtOAc/hexane 1:4); mp: 176–178 °C; ^{1}H NMR (400 MHz, $CDCl_3$): $\delta = 7.40$ (2H, d, $J = 8.7$ Hz, H-2′ and H-6′), 7.25–7.22 (5H, m, ArH″), 6.83 (2H, d, $J = 8.7$ Hz, H-3′ and H-5′), 6.40 (1H, s, H-4), 3.78 (3H, s, CO_2Me), 3.74 (3H, s, $ArOCH_3$), 3.23 (3H, s, NCH_3-1), 2.59 ppm (3H, s, CH_3-6); ^{13}C NMR (100 MHz, $CDCl_3$): $\delta =$ 166.0 (C=O), 160.0, 153.0 (C=O), 151.0 (C=O), 150.3, 149.4, 131.2, 129.6 (2C), 128.0 (2C), 126.3, 121.7 (2C), 114.2 (2C), 109.5, 55.5, 55.4, 52.0, 31.5, 16.5 ppm; IR (CH_2Cl_2): $\tilde{\nu} = 3398$ (N–H), 3052 (C–H), 1778 (C=O), 1730 (C=O), 1648 cm^{-1} (C=O); LRMS (EI): *m/z* (%): 410 (25) $[M+H]^+$; Anal. calcd for $C_{22}H_{22}N_2O_6$: C 64.38, H 5.40, N 6.38%, found: C 64.48, H 5.31, N 6.40%.

A) General method for the preparation of amines 14–16: A solution of the diamine (0.50 mmol) in THF (15 mL) was added to a stirred mixture of **10** (0.20 g, 0.50 mmol) and K_2CO_3 in THF (10 mL) at 25 °C under an argon atmosphere. Stirring was continued for 2 h, by which point TLC analysis indicated complete conversion of starting material to product. The solvent was removed under reduced pressure, and the residue obtained was re-dissolved in CH_2Cl_2 (50 mL). The solution was washed with 5% $NaHCO_3$ (3× 25 mL), brine (50 mL), dried over $MgSO_4$, and filtered. The solvent was reduced in vacuo, and the residue obtained was purified by column chromatography using MeOH/CH_2Cl_2 (1:9) as eluent to give the desired product.

3-[2-(7-Chloroquinolin-4-ylamino)ethylcarbamoyl]-4-(4-methoxyphenyl)-1,6-dimethyl-2-oxo-1,2,3,4-tetrahydropyrimidine-5-carboxylic acid methyl ester (14): White solid (0.25 g, 97%); R_f = 0.35 (MeOH/CH_2Cl_2 1:19); mp: 98–100 °C; ^{1}H NMR (400 MHz, $CDCl_3$): δ = 9.20 (1H, bt, J = 6.4 Hz, N*H*-12″), 8.40 (1H, d, J = 5.6 Hz, H-2″), 7.88 (1H, d, J = 2.0 Hz, H-8″), 7.67 (1H, d, J = 9.2 Hz, H-5″), 7.22 (1H, dd, J = 2.0, 9.2 Hz, H-6″), 7.19 (1H, s, NH-9″), 7.02 (2H, d, J = 9.2 Hz, H-2′ and H-6′), 6.76 (1H, s, H-4), 6.54 (2H, d, J = 9.2 Hz, H-3′ and H-5′), 6.24 (1H, d, J = 5.6 Hz, H-3″), 3.80 (2H, q, J = 5.2 Hz, CH_2-11″), 3.77 (3H, s, CO_2Me), 3.66 (3H, s, ArOCH_3), 3.38 (2H, q, J = 5.2 Hz, CH_2-10″), 3.14 (3H, s, NCH_3), 2.51 ppm (3H, s, CH_3-6); ^{13}C NMR (100 MHz, $CDCl_3$): δ = 165.8 (C=O), 159.0 (C=O), 156.8, 154.4 (C=O), 151.4, 150.7, 148.6, 148.5, 135.4, 131.6, 128.0, 127.8, 127.7, 125.7, 122.2, 117.3, 114.2 (2C), 108.2, 98.4, 55.3, 52.1, 51.5, 46.0, 39.7, 31.6, 16.2 ppm; IR (CH_2Cl_2): $\tilde{\nu}$ = 3320 (N–H), 2987 (C–H), 1765 (C=O), 1693 (C=O), 1638 cm^{-1} (C=O); HRMS-FAB: m/z $[M+H]^+$ calcd for $C_{27}H_{28}ClN_5O_5$: 537.17790, found 537.17355; Anal. calcd for $C_{27}H_{28}ClN_5O_5$: C 60.28, H 5.25, N 13.02%, found: C 60.08, H 5.20, N 12.98%.

3-[3-(7-Chloroquinolin-4-ylamino)propylcarbamoyl]-4-(4-methoxyphenyl)-1,6-dimethyl-2-oxo-1,2,3,4-tetrahydropyrimidine-5-carboxylic acid methyl ester (15): White solid (0.21 g, 78%); R_f = 0.35 (MeOH/CH_2Cl_2 1:19); mp: 81–83 °C; ^{1}H NMR (400 MHz, $CDCl_3$) δ = 9.08 (1H, t, J = 5.6 Hz, N*H*-13″), 8.48 (1H, d, J = 5.6 Hz, H-2″), 7.95 (1H, d, J = 2.4 Hz, H-8″), 7.87 (1H, d, J = 9.2 Hz, H-5″), 7.35 (1H, dd, J = 2.4, 9.2 Hz, H-6″), 7.15–6.74 (5H, m, (4×) Ar-*H*′ and H-4), 6.39 (1H, d, J = 5.6 Hz, H-3″), 6.11 (1H, bt, J = 5.6 Hz, N*H*-9″), 3.69 (3H, s, CO_2Me), 3.67 (3H, s, ArOCH_3), 3.50–3.24 (4H, m, CH_2-10″ and CH_2-12″), 3.16 (3H, s, NCH_3), 2.54 (3H, s, CH_3-6) and 1.97–1.86 ppm (2H, m, CH_2-11″); ^{13}C NMR (100 MHz, $CDCl_3$) δ = 164.7 (C=O), 158.3 (C=O), 153.9, 153.2 (C=O), 150.6, 149.1, 147.9, 147.5, 134.0, 130.8, 127.25, 126.5 (2C), 124.3, 120.7, 116.4, 113.3 (2C), 106.9, 97.6, 54.2, 50.8, 50.1, 38.7, 36.6, 30.3, 27.6, 15.0 ppm; IR (CH_2Cl_2): $\tilde{\nu}$ = 3320 (N–H), 2941 (C–H), 1730 (C=O), 1697 (C=O), 1635 cm^{-1} (C=O); HRMS-FAB: m/z $[M+H]^+$ calcd for $C_{28}H_{30}ClN_5O_5$: 551.19219, found: 551.19355; Anal. calcd for $C_{28}H_{30}ClN_5O_5$: C 60.92, H 5.48, N 12.69%, found: C 60.80, H 5.41, N 12.63%.

3-[4-(7-Chloroquinolin-4-ylamino)butylcarbamoyl]-4-(4-methoxyphenyl)-1,6-dimethyl-2-oxo-1,2,3,4-tetrahydropyrimidine-5-carboxylic acid methyl ester (16): White solid (0.19, 70%); R_f = 0.26 (MeOH/CH_2Cl_2 1:19); mp: 81–83 °C; ^{1}H NMR (400 MHz, $CDCl_3$): δ = 8.90 (1H, bt, J = 6.0 Hz, N*H*-14″), 8.49 (1H, d, J = 5.2 Hz, H-2″), 7.94 (1H, d, J = 2.0 Hz, H-8″), 7.79 (1H, d, J = 8.8 Hz, H-5″), 7.30 (1H, dd, J = 2.0, 8.8 Hz, H-6″), 7.15–6.76 (5H, m, (4×) Ar*H*′ and H-4), 6.38 (1H, d, J = 5.2 Hz, H-3″), 5.51 (1H, bs, N*H*-9″), 3.73 (3H, s, CO_2Me), 3.72 (3H, s, ArOCH_3), 3.60–3.33 (4H, m, CH_2-10″ and CH_2-13″), 3.16 (3H, s, NCH_3), 2.53 (3H, s, CH_3-6) and 1.81–1.72 ppm (4H, m, CH_2-11″ and CH_2-12″); ^{13}C NMR (100 MHz, $CDCl_3$): δ = 166.0 (C=O), 159.5 (C=O), 154.5, 154.4 (C=O), 151.9, 150.2, 149.0, 148.8, 135.1, 132.2, 128.6, 127.8 (2C), 125.5, 121.7, 117.0, 114.2 (2C), 108.0, 99.2, 55.4, 52.0, 51.2, 43.2, 40.3, 31.5, 27.8, 25.8, 16.2 ppm; IR (CH_2Cl_2): $\tilde{\nu}$ = 3320 (N–H), 3057 (C–H), 1770 (C=O), 1703 (C=O), 1635 cm^{-1} (C=O); HRMS-FAB: m/z $[M+1]^+$ calcd for $C_{29}H_{32}ClN_5O_5$: 565.20920, found: 565.20485; Anal. calcd for $C_{29}H_{32}ClN_5O_5$: C 61.53, H 5.70, N 12.37%, found: C 61.52, H 5.65, N 12.27%.

B) General procedure for the preparation of citrate salts 17–19: Citric acid (0.27 g, 1.301 mmol) dissolved in acetone (2 mL) was added dropwise to a solution of **14–16** (1.301 mmol) in acetone (20 mL). The reaction mixture was allowed to stand for 24 h at 5 °C, during which a precipitate formed. The precipitate was filtered and recrystallised from $CHCl_3$ to yield the desired product. Owing to the poor solubility of salts **17–19** in $CDCl_3$ and [D_6]DMSO, the resolution of many signals was decreased, resulting in inaccurate descriptors (e.g. broad s instead of d). However, the integration and position of the citrate protons, required for characterisation of the salts, remain determinable throughout.

3-[2-(7-Chloroquinolin-4-ylamino)ethylcarbamoyl]-4-(4-methoxyphenyl)-1,6-dimethyl-2-oxo-1,2,3,4-tetrahydropyrimidine-5-carboxylic acid methyl ester; compound with 3-carboxy-3-hydroxypentanedioic acid (17): White powder (0.55 g, 57%); mp: 102–108 °C; ^{1}H NMR (300 MHz, $CDCl_3$): δ = 12.34 (3H, bs, COO*H*), 9.22 (1H, bs, NH-12″), 8.75 (1H, bs, H-2″), 8.22 (1H, bs, H-8″), 7.87 (1H, bd, J = 8.7 Hz, H-5″), 7.73 (1H, bs, H-3″), 7.18 (1H, bs, H-6″), 7.06 (2H, J = 8.7 Hz, H-6′ and H-2′), 6.71 (1H, s, H-4), 6.66 (2H, J = 8.7 Hz, H-3′ and H-5′), 6.43 (1H, bs, N*H*-9″), 3.66 (3H, bs, CO_2Me-5), 3.71 (3H, bs, ArOCH_3-4′), 3.56 (4H, bs, CH_2-11″ and CH_2-10″), 3.11 (3H, s, NCH_3), 3.01 (2H, d, J = 15.6 Hz, H-1‴ and H-2‴), 2.90 (2H, d, J = 15.3 Hz, H-1‴ and H-2‴), 2.46 (3H, s, CH_3-6), 2.00 ppm (1H, bs, OH); ^{13}C NMR (75 MHz, $CDCl_3$): δ = 179.3, 174.3, 165.7, 159.2, 155.9, 155.1, 153.9, 148.5, 138.9, 138.6, 131.6, 127.4 (2C), 124.1, 119.7, 115.0, 114.0 (2C), 107.9, 97.9, 73.1, 55.2 (2C), 51.8, 51.3, 44.8 (2C), 39.1, 36.2, 31.5, 30.1, 28.3, 16.1 ppm; IR (nujol): $\tilde{\nu}$ = 3275 (N–H), 1700 (C=O), 1606 cm^{-1} (C=O); LRMS (FAB): m/z (%): 730 (45) $[M+H]^+$; Anal. calcd for $C_{33}H_{36}ClN_5O_{12}$: C 53.63, H 5.05, N 9.48%, found: C 54.29, H 4.97, N 9.59%.

3-[3-(7-Chloroquinolin-4-ylamino)propylcarbamoyl]-4-(4-methoxyphenyl)-1,6-dimethyl-2-oxo-1,2,3,4-tetrahydropyrimidine-5-carboxylic acid methyl ester; compound with 3-carboxy-3-hydroxypentanedioic acid (18): White powder (0.55 g, 83%); mp: 75–79 °C; ^{1}H NMR (400 MHz, $CDCl_3$): δ = 12.30 (3H, bs, COO*H*), 9.04 (1H, bs, NH-13″), 8.68 (1H, bs, H-2″), 8.20 (1H, bs, H-8″), 7.98 (1H, bs, H-5″), 7.66 (1H, bs, H-6″), 7.19 (1H, bs, N*H*-9″), 7.01 (2H, J = 8.8 Hz, H-6′ and H-2′), 6.75 (2H, J = 8.8 Hz, H-3′ and H-5′), 6.72 (1H, s, H-4), 6.38 (1H, bs, H-3″), 3.71 (3H, bs, CO_2Me-5), 3.70 (3H, bs, ArOCH_3-4′), 3.44 (4H, bs, CH_2-12″ and CH_2-10″), 3.13 (3H, s, N-CH_3), 3.00 (2H, d, J = 14.8 Hz, H-1‴ and H-2‴), 2.87 (2H, d, J = 12.4 Hz, H-1‴ and H-2‴), 2.49 (3H, s, CH_3-6), 1.95 (2H, bs, CH_2-11″), 2.05 ppm (1H, bs, O*H*); ^{13}C NMR (100 MHz, $CDCl_3$): δ = 178.7, 173.9 (2C), 165.9, 159.3, 155.1, 155.0, 154.1, 148.7, 143.0, 139.1, 138.2, 131.9, 127.5 (2C), 124.4, 119.6, 115.0, 114.1 (2C), 107.9, 97.9, 73.2, 55.3 (2C), 51.9, 51.3, 44.4, 41.2, 38.1, 31.5, 30.9, 28.1, 16.2 ppm; IR (nujol): $\tilde{\nu}$ = 3275 (N–H), 1700 (C=O), 1606 cm^{-1} (C=O); LRMS (FAB): m/z (%): 744 (20) $[M+H]^+$; Anal. calcd for $C_{34}H_{38}Cl\ N_5O_{12}$: C 54.88, H 5.15, N 9.41%, found: C 54.78, H 5.41, N 9.00%.

3-[4-(7-Chloroquinolin-4-ylamino)butylcarbamoyl]-4-(4-methoxyphenyl)-1,6-dimethyl-2-oxo-1,2,3,4-tetrahydropyrimidine-5-carboxylic acid methyl ester; compound with 3-carboxy-3-hydroxypentanedioic acid (19): White powder (0.55 g, 95%); mp: 71–73 °C; ^{1}H NMR (400 MHz, $CDCl_3$): δ = 12.22 (3H, bs, COO*H*), 8.95 (1H, bs, N*H*-14″), 8.78 (1H, bd, J = 5.6 Hz, H-2″), 8.16 (1H, bs, H-8″), 8.07 (1H, bd, J = 8.0 Hz, H-5″), 7.62 (1H, bs, H-6″), 7.13 (2H, J =

8.8 Hz, H-6′ and H-2′), 7.11 (1H, bs, N*H*-9′′), 6.77 (1H, s, H-4), 6.76 (2H, $J=8.8$ Hz, H-3′ and H-5′), 6.29 (1H, bd, $J=5.6$ Hz, H-3′′), 3.72 (3H, bs, CO_2Me-5), 3.71 (3H, bs, Ar-OCH_3-4′), 3.33 (2H, bs, CH_2-10′′), 3.15 (3H, s, NCH_3), 3.03 (2H, d, $J=14.4$ Hz, H-1′′′ and H-2′′′), 2.92 (2H, d, $J=15.2$ Hz, H-1′′′ and H-2′′′), 2.51 (3H, s, CH_3-6), 1.78 (2H, bs, CH_2-13′′), 1.69 (4H, bs, CH_2-11′′ and CH_2-12′′), 2.01 ppm (1H, bs, OH); ^{13}C NMR (100 MHz, $CDCl_3$): $\delta=179.3$, 174.3, 165.9, 159.3, 155.0, 154.5, 154.3, 148.7, 142.8, 138.9, 138.4, 132.0, 127.5 (2C), 127.1, 124.8, 119.6, 115.0, 114.1 (2C), 107.9, 97.7, 73.2, 55.3 (2C), 51.9, 51.1, 44.9, 43.7, 40.1, 31.5, 30.9, 27.5, 25.0, 16.1 ppm; IR (nujol): $\tilde{\nu}=3275$ (N–H), 1700 (C=O), 1606 cm^{-1} (C=O); LRMS (FAB): *m/z* (%): 758 (70) $[M+H]^+$; Anal. calcd for $C_{35}H_{40}ClN_5O_{12}$: C 55.45, H 5.32, N 9.24%, found: C 55.51, H 5.40, N 9.00%.

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Keywords: drug design · quinoline-containing antimalarials · dihydropyrimidinones · reversing agents · sensitizers

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