Supplementary Material (ESI) for Organic & Biomolecular Chemistry

SUPPORTING INFORMATION

APPLICATION OF MULTICOMPONENT REACTIONS TO ANTIMALARIAL DRUG DISCOVERY. PART 4: ANTIPLASMODIAL, β-HAEMATIN INHIBITION, ANTITRYPANOSOMAL AND CYTOTOXIC ACTIVITY OF NOVEL 4-AMINOQUINOLINE 2-IMIDAZOLINES

Chitalu C. Musonda,[a] Kanyile Ncokazi,[a] Tim J. Egan,[a] Vanessa Yardley,[b] Renata C. Carvalho de Souza[c] and Kelly Chibale[a,d*]

[a]Department of Chemistry and [d]Institute of Infectious Disease and Molecular Medicine, University of Cape Town, Rondebosch 7701, South Africa.
[b]Department of Infectious & Tropical Diseases, London School of Hygiene and Tropical Medicine, Keppel Street, London, WC1E 7HT, UK
[c]Centro de Pesquisas René Rachou, FIOCRUZ, 30190-002 Belo Horizonte, Brazil

1. General Procedures

2. Synthetic Procedure and Characterzation of 6-8, 10-12 and 14-28

1. GENERAL PROCEDURES

Proton nuclear magnetic resonance (^{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 (δ = 0.00). Carbon-13 nuclear magnetic resonance (^{13}C NMR) spectra were recorded at 75 MHz 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 lactams respectively. Mass spectra were recorded by means of a VG micromass 16 F spectrometer at 70 eV with accelerating voltage 4 kV. Accurate masses were determined using a VG-70E spectrometer and VG (Micromass) 70-SE magnetic sector mass spectrometer. Infrared spectra were measured in solution form using chloroform on a satellite FT-IR spectrophotometer. Melting points were determined on a Reichert-Jung Thermovar (temperature range 0 – 350 ^{o}C) on cover slips and are uncorrected. Preparative layer chromatography (p.l.c) was performed on silica 60 F_{254} coated glass plates. Reactions were monitored by thin-layer chromatography (TLC) using silica gel coated plates, and visualized under an ultra-violet lamp. HPLC was performed at ambient temperature on a Thermo Separations Products® instrument, fitted with an Xtera® C_{18} 5 μm, 4.6 x 150 mm column and operated on a uv detector. HPLC-grade acetonitrile and ultrafiltered waster were used as the eluting solvents at a concentration of 30% CH_3CN:70% H_2O in the presence of a 25 mM phosphate buffer.

2. Synthetic Procedures and Characterization

2.1 General Procedure for the synthesis of diamines 6 – 8
A mixture of 4,7-dichloroquinoline (0.98 g, 4.9 mmol) and the diamines **3 – 5** (24.7 mmol, 5 eq) was heated at 80 ^{o}C for 1 h without stirring and then at 135 ^{o}C for 3 h with stirring. The reaction mixture was cooled to room temperature, basified with 10% NaOH (30 ml), extracted with EtOAc (3 x 50 ml) and dried (Na_4SO_4). The solvent was removed *in vacuo* and the residue recrystallised from EtOAc to afford the diamine in high yield.

***N*-(2-Aminoethyl)-7-chloroquinolin-4-amine,**[1] **(6)**
Yellow crystals (1.0 g, 100%); m.p. 115 – 117 ^{o}C; R_f(NH_3:MeOH 1:49) 0.20; δ_H(300 MHz, CD_3OD) 8.35 (d, 1H, *J* 5.4, H-2), 8.1 (d, 1H, *J* 9.0, H-5), 7.77 (d, 1H, *J* 2.1, H-8), 7.38 (dd, 1H, *J* 2.1, 9.0, H-6), 6.6 (d, 1H, *J* 5.4, H-3), 3.43 (t, 2H, *J* 6.0, ArNCH_2), 2.97 (t, 2H, *J* 6.0, NCH_2); δ_C(75 MHz, $CDCl_3$); δ_C (75 MHz, CD_3OD) 152.9, 150.5, 134, 128.9, 126.1, 125.2, 122, 117, 98, 53.6, 46.1.

***N*-(3-Aminopropyl)-7-chloroquinolin-4-amine,**[1] **(7)**
Yellow crystals (1.0 g, 96%), m.p. 124 – 127 ^{o}C; R_f(NH_3:MeOH 1:49) 0.21; δ_H(300 MHz, CD_3OD) 8.33 (d, 1H, *J* 5.4, H-2), 8.12 (d, 1H, *J* 9.0, H-5), 7.75 (d, 1H, *J* 2.1, H-8), 7.42 (dd, 1H, *J* 2.1, 9.0, H-6), 6.62 (d, 1H, *J* 5.4, H-3), 3.44 (t, 2H, *J* 6.0, ArNCH_2), 2.98 (t, 2H, *J* 6.0, NCH_2), 1.89 (m, 2H, CH_2); δ_C(75 MHz, $CDCl_3$); δ_C (75 MHz, CD_3OD) 152.9, 150.5, 134, 128.9, 126.4, 125.1, 122.2, 117, 98.8, 43.6, 41.4, 30.1.

***N*- (4-Aminobutyl)-7-chloroquinolin-amine**,[1] **(8)**
Yellow prisms (1.26 g, 96%), m.p. 45 – 47 ^{o}C; R_f(NH_3:MeOH 1:49) 0.22; δ_H(300 MHz, CD_3OD) 8.48 (d, 1H, *J* 5.4, H-2), 7.94 (d, 1H, *J* 9.0, H-5), 7.69 (d, 1H, *J* 2.1, H-8), 7.27 (dd, 1H, *J* 2.1, 9.0, H-6), 6.33 (d, 1H, *J* 5.4, H-3), 3.39 (t, 2H, *J* 6.0, ArNCH_2(CH_2)$_3$N), 3.04 (t, 2H, *J* 6.0, ArN(CH_2)$_3$CH_2N), 1.89 (m, 2H, ArNCH$_2$CH_2(CH_2)$_2$N), 1.62 (m, 2H, ArN(CH_2)$_2$C$H_2$$CH_2$$NH_2$); δ_C(75 MHz, CD_3OD) 152.9, 150.5, 134.0, 128.9, 124.9, 126.6, 122.0, 117.0, 98.8, 43.6, 41.4, 30.1, 28.9.

2.2 Methyl 2-amino-2-phenylacetate,[2] **(10)**
A suspension of phenylglycine **9** (1.8 g, 11.9 mmol) in MeOH (50 ml) was cooled to 0 ^{o}C in an ice-water bath and $SOCl_2$ added dropwise over 10 min. The clear solution was elevated to ambient temperature and stirred for 2 h, continuously evaporated with DCM and redissolved in MeOH. K_2CO_3 (3.29 g, 23.8 mmol) was added in one portion and the suspension stirred for 15 min, filtered, dried ($MgSO_4$) and concentrated *in vacuo* to afford the aminoester as a white amorphous powder (1.96 g, 100%); R_f(DCM: MeOH 1:9) 0.23; δ_H(300 MHz, $CDCl_3$) 7.49 (m, 5H, 5 x ArH), 5.20 (s, 1H, ArC*H*), 3.81 (s, 3H, OCH_3); δ_C(75 MHz, $CDCl_3$) 161.0, 135.7, 129.0 (2C), 128.4, 127.0 (2C), 54.9, 52.8.

2.3 Methyl 2-formamido-2-phenylacetate,[3] **(11)**

A cooled solution of Ac_2O:HOOCH (1:1.1) was added to a suspension of the amino acetate **10** (11.76 g, 66 mmol) in anhydrous DCM (100 ml), and the mixture refluxed for 2 h. Upon cooling, the solvent was continuously removed until a pale yellow oil formed. The oil was concentrated under reduced pressure and without further purification; it was used in the next step without further purification. Yellow oil, (12.75 g, 100%); R_f(EtOAc:Hex 1:2) 0.22; δ_H(300 MHz, $CDCl_3$) 8.20 (s, 1H, NC*H*O), 7.45 (m, 5H, 5 x ArH), 5.24 (s, 1H, ArC*H*), 3.80 (s, 3H, OCH_3); δ_C(75 MHz, $CDCl_3$) 171.0, 160.1, 136.0, 129.2 (2C), 128.6, 127.1 (2C), 55.0, 53.0.

2.4 Methyl 2-isocyano-2-phenylacetate,[3] **(12)**

A stirred solution of **11** (11.5 g, 65.6 mmol) and Et_3N (45.7 ml, 328.3 mmol) in DCM (250 ml) was cooled to –25 ^oC and $POCl_3$ (7.35 ml, 78.8 mmol) added dropwise. The reaction mixture was stirred at –25 ^oC for an additional 2.5 hours, then a solution of $NaHCO_3$ (20% w/v) was added dropwise so as to maintain the reaction temperature at –25 ^oC. After 3 hours of stirring at low temperature, the reaction mixture was raised to room temperature and stirring continued for 2 h. The aqueous layer was separated and extracted with DCM (2 x 100 ml); the combined organic phases were washed with brine, dried ($MgSO_4$) and evaporated. Column chromatography on SiO_2 gel (eluent EtOAc:Hex 1:4) afforded the isocyanide upon evaporation of the solvent as a yellow oil (2.3 g, 20%); R_f(EtOAc:Hex 1:2) 0.22; IR ν_{max}(Film)/ cm^{-1} 3024 (Ar CH), 2145 (NC), 1712 (C=O), 1661 (Ar C=C); δ_H(300 MHz, $CDCl_3$) 7.43 (m, 5H, 5 x ArH), 5.4 (s, 1H, ArC*H*), 3.79 (s, 3H, OCH_3).

3.0 General Procedure for the parallel synthesis of Imidazolines 14 – 28

A solution of the amine (0.8 mmol) was condensed with aldehyde (0.9 mmol, 1.1 eq) in MeOH (8 ml) at 45 ^oC for 30 min, then isocyanoacetate **12** (0.9 mmol) was added to each of the reaction mixtures. The reactions were stirred for 2 h, after which TLC indicated complete conversion into products. The solvent was removed *in vacuo* and the products purified by column chromatography on SiO_2 gel eluting with 0 – 10% MeOH/DCM.

3.1 *Methyl*-1,1-(2-(7-*chloroquinolin*-4-*ylamino)ethyl*)-4-*phenyl*-4,5-*dihydro*-1*H*-*imidazole*-4-*carboxylate*, 14

Pale yellow foam (290 mg, 84%); R_f(MeOH:DCM 1:9) 0.25; IR ν_{max}($CHCl_3$)/cm^{-1} 3418 (NH), 3042 (ArCH), 2954 (aliphatic CH), 1728 (C=O), 1659 (C=C), 1580 (C=N); δ_H(300 MHz, $CDCl_3$) 8.50 (d, 1H, *J* 5.7, H-2), 7.98 (d, 1H, *J* 2.1, H-8), 7.70 (d, 1H, *J* 9.0, H-5), 7.40 (dd, 1H, *J* 2.1, 9.0, H-6), 7.38 – 7.20 (m, 5H, 5 x PhH), 6.98 (s, 1H, H-2′), 6.30 (d, 1H, *J* 5.7, H-3), 4.40 (d, 1H, *J* 9.6, H-5′α), 3.69 (s, 3H, OCH_3), 3.30 (d, 1H, *J* 9.6, H-5′β), 3.29 – 3.15 (m, 4H, ArNCH_2CH_2N); δ_C(75 MHz, $CDCl_3$) 173.3, 156.7, 154.0, 151.0, 149.0, 148.0, 142.0, 134.0, 128.6 (2C), 127.6, 125.5, 125.3 (2C), 121.0, 117.0, 99.0, 80.1, 57.6, 53.0, 46.6, 42.8; HRMS (EI) 408.13406, (M^+ $C_{22}H_{21}{}^{35}ClN_4O_2$ requires 408.13530); HPLC Purity: 92%; t_R' = 2.43 min.

3.2 *Methyl*-1-(2-(7-*chloroquinolin*-4-*ylamino*)*ethyl*)-5-*methyl*-4-*phenyl*-4,5-*dihydro*-1*H*-i*midazole*-4-*carboxylate*, 15

Yellow oil (276 mg, 82%); R_f (MeOH:DCM 1:9) 0.25; IR ν_{max}($CHCl_3$)/cm^{-1} 3449 (NH), 3013 (ArCH), 2953 (aliphatic CH), 1728 (C=O), 1650 (C=C), 1581 (C=N); δ_H(300 MHz, $CDCl_3$) (major/minor) 8.51/8.46 (d, 1H, *J* 5.7, H-2), 7.93/7.90 (d, 1H, *J* 2.1, H-8), 7.78/7.65 (d, 1H, *J* 9.0, H-5), 7.43/7.32 (dd, 1H, *J* 2.1, 9.0, H-6), 7.35 – 7.25 (m, 5H/5H, 5 x PhH), 7.04/7.02 (s, 1H, H-2′), 6.44/6.18 (d, 1H, *J* 5.7, H-3), 4.53/4.0 (q, 1H, *J* 6.6, H-5′), 3.69/3.66 (s, 3H, OCH_3), 3.42/3.27 (m, 4H, 2 x ArNCH_2CH_2N), 1.35/0.68 (d, 3H, *J* 6.6, CH_3); δ_C(75 MHz, $CDCl_3$) 174.2, 156.0, 154.1, 149.2, 148.6, 142.6, 137.4, 135.0, 128.3 (2C), 126.4 (2C), 126.1, 125.5, 121.0, 117.2, 98.9, 82.5, 59.8, 52.7, 41.7, 40.5, 14.7; HRMS (EI) 422.15147, (M^+ $C_{23}H_{23}{}^{35}ClN_4O_2$ requires 422.15095); HPLC Purity: 96%; t_R' = 2.40 min.

3.3 *Methyl*-1-(2-(7-*chloroquinolin*-4-*ylamino*)*ethy*l)-5-*ferrocenyl*-4-*phenyl*-4,5-*dihydro*-1*H*-*imidazole*-4-*carboxylate*, 16

Dark brown oil (384 mg, 76%); R_f(MeOH:DCM 1:9) 0.27; IR ν_{max}($CHCl_3$)/cm^{-1} 3422 (NH), 3012 (ArCH), 2953 (aliphatic CH), 1711 (C=O), 1652 (C=C), 1581 (C=C); δ_H(300 MHz, $CDCl_3$) (major/minor) 8.54/8.41 (d, 1H, *J* 5.7, H-2), 7.93/7.91 (d, 1H, *J* 2.1, H-8), 7.76/7.66 (d, 1H, *J* 9.0, H-5), 7.44/7.30 (dd, 1H, *J* 2.1, 9.0, H-6), 7.36 – 7.27 (m, 5H/5H, 5 x PhH), 7.14/7.13 (s, 1H, H-2′), 6.40/6.16 (d, 1H, *J* 5.7, H-3), 5.39/4.81 (s, 1H, H-5′), 4.20/4.00 (m, 9H, Fc*H*), 3.84/3.82 (s, 3H, OCH_3), 3.78/3.76 (m, 2H, ArNCH_2CH_2N), 3.58/3.42 (m, 2H, ArNCH_2CH_2N); δ_C(75 MHz, $CDCl_3$) 174.0, 156.0, 155.0, 151.0, 149.0, 137.2, 137.4, 135.0, 128.6, 127.7 (2C), 126.8 (2C), 125.4, 121.0, 117.0, 98.9, 85.0, 68.0 (5C), 67.0, 66.8 (2C), 66.6 (2C), 65.6, 63.8, 53.0, 45.0; HRMS (EI) 592.13205, (M^+ $C_{32}H_{29}{}^{35}ClFeN_4O_2$ requires 592.13285); HPLC Purity: 98%; t_R' = 2.33 min.

3.4 *Methyl*-1-(2-(7-*chloroquinolin*-4-*ylamino*)*ethyl*)-5-*isobutyl*-4-*phenyl*-4,5-*dihydro*-1*H*-*imidazole*-4-*carboxylate*, 17

The method used for the synthesis of **5.73** was that described in the General Procedure for the synthesis of imidazolines, using 0.3 mmol of amine. The product was obtained as a brown oil, (65 mg, 86%); R_f(MeOH:DCM 1:9) 0.26; IR ν_{max}($CHCl_3$)/cm^{-1} 3411 (NH), 3023 (ArCH), 2935 (aliphatic CH), 1711 (C=O), 1582 (C=N); δ_H(300 MHz, $CDCl_3$) (major/minor) 8.55/8.53 (d, 1H, *J* 5.7, H-2), 7.96/7.93 (d, 1H, *J* 2.1, H-8), 7.74/7.63 (d, 1H, *J* 9.0, H-5), 7.44/7.42 (dd, 1H, *J* 2.1, 9.0, H-6), 7.38 – 7.26 (m, 5H/5H, 5 x PhH), 7.07/7.04 (s, 1H, H-2′), 6.40/6.21 (d, 1H, *J* 5.7, H-3), 3.71/3.69 (s, 3H, OCH_3) 3.60/3.48 (m, 1H, H-5′), 3.38/3.29 (m, 2H, ArNCH_2CH_2N, 3.27/3.25 (m, 2H, ArNCH_2CH_2N), 3.22/3.18 (m, 1H, CH_2C*H*(CH_3)$_2$), 2.5/2.20 (m, 2H, CH_2CH(CH_3)$_2$), 1.09/0.97 (d, 3H, *J* 6.6, CH_3), 0.96/0.42 (d, 3H, *J* 6.6, CH_3); δ_C(75 MHz, $CDCl_3$) 174.2, 156.1, 154.0, 149.2, 148.6, 142.6, 137.4, 135.2, 128.3 (2C), 126.4 (2C), 126.1, 125.5, 121.0, 117.2, 98.9, 84.4, 74.0, 66.1, 51.6, 46.4, 43.0, 21.3, 17.2 (2C); HRMS (EI) 464.19786, (M^+ $C_{26}H_{29}{}^{35}ClN_4O_2$ requires 464.19790); HPLC Purity: 95%; t_R' = 2.46 min.

3.5 *Methyl*-1-(2-(7-*chloroquinolin*-4-*ylamino*)*ethyl*)-5-(2-*furfuryl*)-4-*phenyl*-4,5-*dihydro*-1*H*-*imidazole*-4-*carboxylate* 18

Orange foam (382 mg, 95%); R_f (MeOH:DCM 1:9) 0.27; IR ν_{max}($CHCl_3$)/cm^{-1} 3449 (NH), 3013 (ArCH), 2953 (aliphatic CH), 1728 (C=O), 1650 (C=C), 1581 (C=N); δ_H(300 MHz, $CDCl_3$) (major/minor) 8.51/8.46 (d, 1H, *J* 5.7, H-2), 7.93/7.87 (d, 1H, *J* 2.1, H-8), 7.78/7.65 (d, 1H, *J* 9.0, H-5), 7.43/7.32 (dd, 1H, *J* 2.1, 9.0, H-6), 7.35 – 7.25 (m, 5H/5H, 5 x PhH), 7.19/7.08 (dd, 1H, *J* 1.5, 3.3, H-5″), 7.01/6.97 (s, 1H, H-2′), 6.44/6.30 (d, 1H, *J* 5.7, H-3), 6.28/6.19 (t, 1H, *J* 3.3, H-4″), 6.08/6.00 (dd, 1H, *J* 1.5, 3.3, H-3″), 5.31/5.19 (s, 1H, H-5′), 3.71/3.67 (s, 3H, OCH_3), 3.42/3.25 (m, 4H, 2 x ArNCH_2CH_2N); δ_C(75 MHz, $CDCl_3$) 174.2, 157.5, 156.0, 154.1, 149.2, 148.6, 142.6, 140.4, 137.4, 135.0, 128.3 (2C), 126.4 (2C), 126.1, 125.5, 121.0, 117.2, 110.1, 104.6, 98.9, 82.5, 59.8, 52.7, 41.7, 40.5; HRMS (EI) 474.14603, (M^+ $C_{26}H_{23}{}^{35}ClN_4O_3$ requires 474.14587); HPLC Purity: 99%; t_R' = 2.51 min.

3.6 *Methyl*-1-(3-(7-*chloroquinolin*-4-*ylamino*)*propyl*)-4-*phenyl*-4,5-*dihydro*-1*H*-*imidazole*-4-*carboxylate*, 19

Yellow oil (278 mg, 78%); R_f(MeOH:DCM 1:9) 0.26; IR ν_{max}($CHCl_3$)/cm^{-1} 3229 (NH), 3054 (ArCH), 2986 (aliphatic CH), 1728 (C=O), 1658 (C=C), 1582 (C=N); δ_H(300 MHz, $CDCl_3$) 8.50 (d, 1H, *J* 5.7, H-2), 7.98 (d, 1H, *J* 2.1, H-8), 7.70 (d, 1H, *J* 9.0, H-5), 7.40 (dd, 1H, *J* 2.1, 9.0, H-6), 7.38 – 7.20 (m, 5H, 5 x PhH), 7.00/6.98 (s, 1H, H-2′), 4.4 (d, 1H, *J* 9.6, H-5′α, 3.69 (s, 3H, OCH_3), 3.30 (d, 1H, *J* 9.6, H-5′-β), 3.28 – 3.13 (m, 4H, ArNCH_2CH_2N), 1.70 (m, 2H,

ArNCH$_2$C*H*$_2$CH$_2$N); δ_C(75 MHz, $CDCl_3$) 173.3, 156.7, 154.2, 151.0, 149.0, 148.0, 142.0, 134.0, 128.6 (2C), 127.6, 125.5, 125.3 (2C), 121.0, 117.1, 99.2, 80.1, 57.6, 53.2, 46.6, 42.8, 26.0; HRMS (EI) 422.15023, (M^+ $C_{23}H_{22}{}^{35}ClN_4O_2$ requires 422.15095); HPLC Purity: 92%; t_R' = 2.44 min.

3.7 ***Methyl*-1-(3-(7-*chloroquinolin*-4-*ylamino*)*propyl*)-5-*methyl*-4-*phenyl*-4,5-*dihydro*-1*H*-*imidazole*-4-*carboxylate***, 20**

Pale yellow foam (206 mg, 55%); R_f(MeOH:DCM 1:9) 0.27; IR ν_{max}($CHCl_3$)/cm^{-1} 3449 (NH), 3013 (ArCH), 2953 (aliphatic CH), 1728 (C=O), 1655 (C=C), 1580 (C=N); δ_H(300 MHz, $CDCl_3$) (major/minor) 8.54/8.41 (d, 1H, *J* 5.7, H-2), 7.93/7.91 (d, 1H, *J* 2.1, H-8), 7.76/7.66 (d, 1H, *J* 9.0, H-5), 7.44/7.30 (dd, 1H, *J* 2.1, 9.0, H-6), 7.36 – 7.27 (m, 5H/5H, 5 x PhH), 7.12/7.10 (s, 1H, H-2′), 6.40/6.16 (d, 1H, *J* 5.7, H-3), 4.56/3.97 (q, 1H, *J* 6.6, CH_3C*H*), 3.69/3.67 (s, 3H, OC*H*$_3$), 3.4/3.25 (m, 4H, ArNC*H*$_2$C*H*$_2$N), 2.0/1.92 (quint, 2H, *J* 7.2, ArNCH$_2$C*H*$_2$CH$_2$), 1.36/0.64 (d, 3H, *J* 6.6, C*H*$_3$); δ_C(75 MHz, $CDCl_3$) 174.0, 156.0, 151.0, 149.0, 148.8, 142.7, 137.2, 135, 128.3 (2C), 126.1, 126.0 (2C), 125.4, 121.0, 117.0, 98.9, 82.7, 59.8, 52.9, 41.8, 40.3, 27.5, 14.5; HRMS (EI) 436.16577, (M^+ $C_{24}H_{25}{}^{35}ClN_4O_2$ requires 436.16660); HPLC Purity: 96%; t_R' = 2.38 min.

3.8 ***Methyl*-1-(3-(7-*chloroquinolin*-4-*ylamino*)*propyl*)-5-*ferrocenyl*-4-*phenyl*-4,5-*dihydro*-1*H*-*imidazole*-4-*carboxylate***, 21**

Brown foam (397 mg, 77%); R_f(MeOH:DCM 1:9) 0.30; IR ν_{max}($CHCl_3$)/cm^{-1} 3430 (NH), 3037 (ArCH), 2953 (aliphatic CH), 1725 (C=O), 1642 (C=C), 1582 (C=N); δ_H(300 MHz, $CDCl_3$) (major/minor) 8.55/8.43 (d, 1H, *J* 5.7, H-2), 7.94/7.90 (d, 1H, *J* 2.1, H-8), 7.77/7.63 (d, 1H, *J* 9.0, H-5), 7.41/7.39 (dd, 1H, *J* 2.1, 9.0, H-6), 7.34 – 7.24 (m, 5H/5H, 5 x PhH), 7.08/7.06 (s, 1H, H-2′), 6.41/6.20 (d, 1H, *J* 5.7, H-3), 5.38/4.79 (s, 1H, H-5′), 4.18/4.0 (m, 9H, Fc*H*), 3.83/3.80 (s, 3H, OC*H*$_3$), 3.79/3.77 (m, 2H, ArNC*H*$_2$CH$_2$N), 3.57/3.44 (m, 2H, ArNCH$_2$CH$_2$C*H*$_2$N), 2.1/1.80 (m, 2H, ArNCH$_2$C*H*$_2$CH$_2$N); δ_C(75 MHz, $CDCl_3$) 174.0, 156.0, 155.0, 151.0, 149.0, 137.3, 137.2, 135.2, 128.4, 127.3 (2C), 126.5 (2C), 125.3, 121.0, 117.1, 99.0, 85.3, 68.2 (5C), 67.3, 66.7 (2C), 66.5 (2C), 65.7, 63.7, 53.0, 45.0, 26.6; HRMS (EI) 606.14566, (M^+ $C_{33}H_{31}{}^{35}ClN_4O_2$ requires 606.14580); HPLC Purity: 92%; t_R' = 2.37 min.

3.9 ***Methyl*-1-(3-(7-*chloroquinolin*-4-*ylamino*)*propyl*)-5-*isobutyl*-4-*phenyl*-4,5-*dihydro*-1*H*-*imidazole*-4-*carboxylate***, 22**

Cream white foam (238 mg, 56%); R_f(MeOH:DCM 1:9) 0.29; IR ν_{max}($CHCl_3$)/cm^{-1} 3427 (NH), 2963 (aliphatic CH), 1713 (C=O), 1642 (C=C), 1583 (C=N); δ_H(300 MHz, $CDCl_3$) (major/minor) 8.55/8.53 (d, 1H, *J* 5.7, H-2), 7.96/7.93 (d, 1H, *J* 2.1, H-8), 7.74/7.63 (d, 1H, *J* 9.0, H-5), 7.44/7.42 (dd, 1H, *J* 2.1, 9.0, H-6), 7.38 – 7.26 (m, 5H/5H, 5 x PhH), 7.06/7.04 (s, 1H, H-2′), 6.40/6.21 (d, 1H, *J* 5.7, H-3), 3.58/3.42 (m, 1H, H-5′), 3.68/3.37 (s, 3H, OC*H*$_3$), 3.38/3.29 (m, 2H, ArNC*H*$_2$(CH$_2$)$_3$N), 3.27/3.25 (m, 2H, ArN(CH$_2$)$_3$C*H*$_2$N), 3.22/3.18 (m, 1H, C*H*(CH$_3$)$_2$), 2.5/2.20 (m, 2H, C*H*$_2$CH), 2.05/1.90 (m, 2H, ArNCH$_2$C*H*$_2$CH$_2$N), 1.09/0.97 (d, 3H, *J* 6.6, C*H*$_3$), 0.96/0.42 (d, 3H, *J* 6.6, C*H*$_3$); δ_C(75 MHz, $CDCl_3$) 174.2, 156.0, 154.1, 149.2, 148.6, 142.6, 137.4, 135.0, 128.3 (2C), 126.4 (2C), 126.1, 125.5, 121.0, 117.2, 98.9, 84.4, 74.0, 66.1, 51.6, 43.0, 40.0, 28.0, 26.8, 21.3, 17.2; HRMS (EI) (M^+ $C_{27}H_{31}{}^{35}ClN_4O_2$ requires 478.21355); HPLC Purity: 99%; t_R' = 2.42 min.

3.10 ***Methyl-1-(3-(7-chloroquinolin-4-ylamino)propyl)-5-(2-furfuryl)-4-phenyl-4,5-dihydro*-1*H*-*imidazole*-4-*carboxylate* 23**

Pale yellow foam (396 mg, 96%); R_f(MeOH:DCM 1:9) 0.27; IR ν_{max}($CHCl_3$)/cm^{-1} 3449 (NH), 3013 (ArCH), 2953 (aliphatic CH), 1728 (C=O), 1655 (C=C), 1580 (C=N); δ_H(300 MHz, $CDCl_3$) (major/minor) 8.54/8.41 (d, 1H, *J* 5.7, H-2), 7.93/7.91 (d, 1H, *J* 2.1, H-8), 7.76/7.66 (d, 1H, *J* 9.0, H-5), 7.44/7.36 (dd, 1H, *J* 2.1, 9.0, H-6), 7.30 – 7.27 (m, 5H/5H, 5 x PhH), 7.19/7.10 (dd, 1H, *J* 1.5, 3.6, H-5″) 7.02/6.88 (s, 1H, H-2′), 6.40/6.16 (d, 1H, *J* 5.7, H-3), 6.28/6.19 (t, 1H, *J* 3.6, H-4″), 6.12/6.00 (dd, 1H, *J* 1.5, 3.6, H-3″), 5.33/5.14 (s, 1H, H-5′), 3.69/3.67 (s, 3H, OC*H*$_3$), 3.40/3.25 (m, 4H/4H, ArNC*H*$_2$C*H*$_2$N), 2.01/1.92 (quint, 2H, *J* 7.2, ArNCH$_2$C*H*$_2$CH$_2$); δ_C(75 MHz, $CDCl_3$) 174.0, 157.5, 156.0, 151.0, 149.0, 148.8, 142.7, 140.1, 137.2, 135, 128.3 (2C), 126.1, 126.0 (2C), 125.4, 121.0, 117.0, 110.5, 104.9, 98.9, 82.7, 59.8, 52.9, 41.8, 40.3; HRMS (EI) 488.16109, (M^+ $C_{27}H_{25}{}^{35}ClN_4O_3$ requires 488.16152); HPLC Purity: 95%; t_R' = 2.57 min.

3.11 ***Methyl*-1-(4-(7-*chloroquinolin*-4-*ylamino*)*butyl*)-4-*phenyl*-4,5-*dihydro*-1*H*-*imidazole*-4-*carboxylate***, 24**

Pale orange foam (250 mg, 67%); R_f(MeOH:DCM 1:9) 0.26; IR ν_{max}($CHCl_3$)/cm^{-1} 3430 (NH), 3039 (Ar CH), 2952 (aliphatic CH), 1728 (C=O), 1640 (C=C), 1582 (C=N); δ_H(300 MHz, $CDCl_3$) 8.50 (d, 1H, *J* 5.7, H-2), 7.98 (d, 1H, *J* 2.1, H-8), 7.70 (d, 1H, *J* 9.0, H-5), 7.40 (dd, 1H, *J* 2.1, 9.0, H-6), 7.38 – 7.20 (m, 5H, 5 x PhH), 6.98 (s, 1H, H-2′), 4.4 (d, 1H, *J* 9.6, H-5′α), 3.69 (s, 3H, OC*H*$_3$), 3.31 (d, 1H, *J* 9.6, H-5′β), 3.28 – 3.17 (m, 4H, ArNC*H*$_2$(CH$_2$)$_2$C*H*$_2$N), 1.82 (m, 2H, ArNCH$_2$C*H*$_2$(CH$_2$)$_2$N), 1.62 (m, 2H, ArN(CH$_2$)$_2$C*H*$_2$CH$_2$N); δ_C(75 MHz, $CDCl_3$) 173.3, 156.7, 154.1, 151.0, 149.0, 148.0, 142.0, 134.0, 128.6 (2C), 127.6, 125.5, 125.3 (2C), 121.0, 117.0, 99.0, 80.1, 57.6, 53.0, 46.6, 42.8, 30.3, 26.0; HRMS (EI) 436.16465, (M^+ $C_{24}H_{24}{}^{35}ClN_4O_2$ requires 436.16660); HPLC Purity: 94%; t_R' = 2.39 min.

3.12 ***Methyl*-1-(4-(7-*chloroquinolin*-4-*ylamino*)*butyl*)-5-*methyl*-4-*phenyl*-4,5-*dihydro*-1*H*-*imidazole*-4-*carboxylate*, 25**

Pale yellow foam (165 mg, 43%); R_f(MeOH:DCM 1:9) 0.27; IR ν_{max}($CHCl_3$)/cm^{-1} 3428 (NH), 3030 (ArCH, 2951 (aliphatic CH), 1733 (C=O), 1649 (C=C), 1580 (C=N); δ_H(300 MHz, $CDCl_3$) (major/minor) 8.54/8.41 (d, 1H, *J* 5.7, H-2), 7.93/7.91 (d, 1H, *J* 2.1, H-8), 7.76/7.66 (d, 1H, *J* 9.0, H-5), 7.42/7.29 (dd, 1H, *J* 2.1, 9.0, H-6), 7.36 – 7.27 (m, 5H/5H, 5 x PhH), 7.24/7.1 (s, 1H, H-2′), 6.40/6.16 (d, 1H, *J* 5.7, H-3’), 4.56/3.97 (q, 1H, *J* 6.6, H-5′), 3.69/3.67 (s, 3H, OC*H*$_3$), 3.40/3.25 (m, 4H, ArNC*H*$_2$NC*H*$_2$N), 2.0/1.92 (quint, 2H, *J* 7.2, ArNCH$_2$C*H*$_2$(CH$_2$)$_2$N), 1.71/1.63 (m, 2H, ArN(CH$_2$)$_2$C*H*$_2$CH$_2$N), 1.36/0.64 (d, 3H, *J* 6.6, CHC*H*$_3$); δ_C(75 MHz, $CDCl_3$) 174.0, 156.0, 151.0, 149.0, 148.8, 142.7, 137.2, 135.1, 128.3 (2C), 126.0 (2C), 126.1, 125.4, 121.0, 117.1, 98.9, 82.7, 59.8, 52.9, 41.8, 40.3, 30.0, 26.8, 14.5; HRMS (EI) 450.18224, (M^+ $C_{25}H_{27}{}^{35}ClN_4O_2$ requires 450.18225); HPLC Purity: 95%; t_R' = 2.42 min.

3.13 ***Methyl*-1-(4-(7-*chloroquinolin*-4-*ylamino*)*butyl*)-5-*ferrocenyl*-4-*phenyl*-4,5-*dihydro*-1*H*-*imidazole*-4-*carboxylate***, 26**

Light brown foam (392 mg, 74%); R_f(MeOH:DCM 1:9) 0.30; IR ν_{max}($CHCl_3$)/cm^{-1} 3428 (NH), 3034 (ArCH), 2953 (aliphatic CH), 1728 (C=O), 1651 (C=C), 1581 (C=N); δ_H(300 MHz, $CDCl_3$) (major/minor) 8.53/8.44 (d, 1H, *J* 5.7, H-2), 7.98/7.94 (d, 1H, *J* 2.1, H-8), 7.71/7.67 (d, 1H, *J* 9.0, H-5), 7.41/7.37 (dd, 1H, *J* 2.1, 9.0, H-6), 7.35 – 7.25 (m, 5H/5H, 5 x PhH), 7.22/7.03 (s, 1H, H-2′), 6.43/6.26 (d, 1H, *J* 5.7, H-3), 5.4/4.8 (s, 1H, H-5′), 4.20/3.80 (m, 9H, Fc*H*), 3.72/3.70 (s, 3H, OC*H*$_3$), 3.57/3.48(m, 2H, ArNC*H*$_2$(CH$_2$)$_3$N), 3.46/3.44 (m, 2H, ArN(CH$_2$)$_3$C*H*$_2$N), 2.10/1.80 (m, 2H, ArNCH$_2$C*H*$_2$(CH$_2$)$_2$N), 1.72/1.68 (m, 2H, ArN(CH$_2$)$_2$C*H*$_2$CH$_2$N); δ_C(75 MHz, $CDCl_3$) 174.0, 156.0, 155.0, 151.0, 149.0, 137.3, 137.2, 135.0, 128.4, 127.3 (2C), 126.5 (2C), 125.3, 121.0, 117.0, 99.0, 85.3, 68.2 (5C), 67.2, 66.7 (2C), 66.5 (2C), 65.7, 63.7, 53.0, 45.0, 27.7, 26.8; HRMS (EI) 620.16403, (M^+ $C_{34}H_{33}{}^{35}ClN_4O_2$ requires 620.16415); HPLC Purity: 97%; t_R' = 2.42 min.

3.14 ***Methyl*-1-(4-(7-*chloroquinolin*-4-*ylamino*)*butyl*)-5-*isobutyl*-4-*phenyl*-4,5-*dihydro*-1*H*-*imidazole*-4-*carboxylate*, 27**

Cream white foam (218 mg, 52%); R_f(MeOH:DCM 1:9) 0.29; IR ν_{max}($CHCl_3$)/cm^{-1} 3422 (NH), 3034 (ArCH), 2955 (aliphatic CH), 1716 (C=O), 1658 (C=C), 1580 (C=N); δ_H(300 MHz, $CDCl_3$) (major/minor) 8.54/8.52 (d, 1H, *J* 5.7, H-2), 7.98/7.94 (d, 1H, *J* 2.1, H-8), 7.70/7.66 (d, 1H, *J* 9.0, H-5), 7.42/7.40 (dd, 1H, *J* 2.1, 9.0, H-6), 7.37 – 7.22 (m, 5H/5H, 5 x PhH), 7.03/7.01 (s, 1H, H-2′), 6.39/6.24 (d, 1H, *J* 5.7, H-3), 3.66/3.64 (s, 3H, OC*H*$_3$), 3.57/3.46 (m, 1H, H-5′),

3.30/3.29 (m, 2H, ArNCH_2(CH_2)$_3$N), 3.27/3.24 (m, 2H, ArN(CH_2)$_3$CH_2N), 3.22/3.18 (m, 1H, CH(CH_3)$_2$), 2.5/2.20 (m, 2H, CH_2CH), 2.03/1.87 (m, 2H, ArNCH$_2$CH_2CH$_2$N), 1.70/1.66 (m, 2H, ArN(CH_2)$_2$CH_2CH$_2$N), 1.09/0.97 (d, 3H, *J* 6.6, CH_3), 0.96/0.42 (d, 3H, *J* 6.6, CH_3); δ_C(75 MHz, $CDCl_3$) 174.2, 156, 154.0, 149.2, 148.6, 142.6, 137.4, 135, 128.3 (2C), 126.4 (2C), 126.1, 125.5, 121.0, 117.2, 98.9, 84.4, 74.0, 66.1, 53.1, 51.6, 43.2, 40.1, 28.0, 26.8, 21.3, 17.2; HRMS (EI) 492.22065, (M^+ $C_{28}H_{33}{}^{35}ClN_4O_2$ requires 492.22920); HPLC Purity: 96%; t_R' = 2.46 min.

3.15 *Methyl*-1-(4-(7-*chloroquinolin*-4-*ylamino*)*butyl*)-5-(2-*furfuryl*)-4-*phenyl*-4,5-*dihydro*-1*H*-*imidazole*-4-*carboxylate* 28

Pale orange foam (422 mg, 95%); R_f(MeOH:DCM 1:9) 0.26; IR ν_{max}($CHCl_3$)/cm^{-1} 3430 (NH), 3039 (Ar CH), 2952 (aliphatic CH), 1728 (C=O), 1640 (C=C), 1582 (C=N); δ_H(300 MHz, $CDCl_3$) 8.50/8.43 (d, 1H, *J* 5.7, H-2), 7.98/7.88 (d, 1H, *J* 2.1, H-8), 7.70/7.63 (d, 1H, *J* 9.0, H-5), 7.40/7.36 (dd, 1H, *J* 2.1, 9.0, H-6), 7.38 – 7.20 (m, 5H/5H, 5 x PhH), 7.19/7.10 (dd, 1H, *J* 1.5, 3.6, H-5″), 6.98/6.92 (s, 1H, H-2′),6.47/6.35 (d, 1H, *J* 5.7, H-3), 6.28/6.19 (t, 1H, *J* 3.6, H-4″), 6.12/6.00 (dd, 1H, *J* 1.5, 3.6, H-3″), 5.33/5.14 (s, 1H, H-5′), 3.66/3.61 (s, 3H, OCH_3), 3.30/3.18 (d, 1H, *J* 9.6, H-5′β), 3.28 – 3.17 (m, 4H, ArNCH_2(CH_2)$_2$CH_2N), 1.82/1.72 (m, 2H, ArNCH$_2$CH_2(CH_2)$_2$N), 1.62/1.45 (m, 2H, ArN(CH_2)$_2$CH_2CH$_2$N); δ_C(75 MHz, $CDCl_3$) 173.3, 157.5, 156.7, 154.1, 151.0, 149.0, 148.0, 142.0, 140.3, 134.0, 128.6 (2C), 127.6, 125.5, 125.3 (2C), 121.0, 117.0, 110.0, 104.5, 99.0, 80.1, 57.6, 53.0, 46.6, 42.8, 30.3, 26.0; HRMS (EI) 502.17189, (M^+ $C_{28}H_{27}{}^{35}ClN_4O_3$ requires 502.17717); HPLC Purity: 98%; t_R' = 2.69 min.

4.0 *In Vitro* Activities of compounds against 3D7 and K1 *P. falciparum*,

Two clones of *P. falciparum* were used: (a) the 3D7 clone of NF54 which is known to be sensitive to all anti-malarials, (b) the K1 strain originating from Thailand that is resistant to chloroquine and pyrimethamine, but sensitive to mefloquine. The cultures were naturally asynchronous (65 – 75% ring stage) and were maintained in continuous log phase growth in RPMI1640 medium supplemented with 5% washed human A+ erythrocytes, 25 mM Hepes, 32 nM $NaHCO_3$, and AlbuMAXII (lipid-rich bovine serum albumin) (GIBCO, Grand Island, NY) (CM). All cultures and assays are conducted at 37 °C under an atmosphere of 5% CO_2 and 5% O_2, with a balance of N_2.

4.1 Drug sensitivity assays

Stock drug solutions were prepared in 100% DMSO (dimethylsulfoxide) at 20 mg/ml unless otherwise suggested by the supplier. The compound was further diluted to the appropriate concentration using complete medium RPMI1640 supplemented with 15 nM cold hypoxanthine and AlbuMAXII.

Assays were performed in sterile 96-well microtitre plates, each plate contained 100 µl of parasite culture (0.5% parasitaemia, 2.5% hematocrit). Each drug was tested in triplicate and parasite growth compared to control and blank (uninfected erythrocytes) wells. After 24 h of incubation at 37 °C, 3.7Bq of [^{3}H]hypoxanthine were added to each well. Cultures were incubated for a further 24 h before they were harvested onto glass-fiber filter mats. The radioactivity was counted using a Wallac Microbeta 1450 scintillation counter. The results were recorded as counts per minute (CPM) per well at each drug concentration, control and blank wells. Percentage inhibition was calculated from comparison to blank and control wells, and IC_{50} values calculated using Microsoft *XL*Fit line fitting software (IDBS, UK).

4.2 Primary screen

The preliminary screen used the 3D7 strain. The compounds were tested at 6 concentrations (30, 10, 3, 1, 0.3, and 0.1 µg/ml). If the compound did not affect parasite growth at 10 µg/ml it was classified as inactive, between 10 and 1 µg/ml, the compound was designated as partially active, and if <1 µg/ml, the compound was classified as active and was further evaluated by three-fold serial dilutions in a repeat test.

4.3 Secondary screen

Both the 3D7 clone and the K1 line were used. The compound was diluted three-fold over at 12 different concentrations with an appropriate starting concentration based on the preliminary screen. The IC_{50} was determined by a sigmoidal dose response analysis using Microsoft *XL*Fit™ (IDBS, UK). For each assay, the IC_{50} and IC_{90} values for each parasite line were determined against the known anti-malarial chloroquin, plus other standard compounds appropriate for the assay.

5.0 β-Haematin Inhibition

This assay is based on the ability of haematin, but not β-haematin to form a low spin complex with aqueous pyridine at pH 7.5. The assay has been fully described in detail elsewhere [1]. Briefly, serial dilutions of the drug solutions were carried out in triplicate in a 96 well plate using a multichannel pipette. Drug concentrations varied from 1-10 equivalents relative to haematin each well containing 10.1 µl in the final mixture. Then, 101.2 µl of haematin stock solution (1.7 mM in 0.1 M NaOH) was added to each well followed by 10.1 µl of 1.0 M HCl. The 58.7 µl of acetate solution (12.9 M, pH 5.0) which was pre-incubated at 60 ^oC was added and the plate was incubated at 60 ^oC for 60 minutes. The mixture was quenched with 80 µl of 30% (v/v) pyridine solution in 20 mM HEPES, pH 7.5 and allowed to settle at room temperature. Then 38 µl of supernatant was transferred to another plate and diluted to 250 µl with 30% (v/v) of pyridine solution (pH 7.5, 20 mM HEPES). The absorbance was read at 405nm using an ASYS UVM 340 plate reader. The IC_{50} values for β-haematin inhibition were determined by fitting the absorbance data to a sigmoidal dose response curve by non-linear least squares fitting using GraphPad Prism software [2].

1. D. De, L.D. Byers, D.J. Krogstad, *J. Heterocyclic Chem*., **1997**, *34*, 315 – 320.
2. T. Kihlberg, F. Karimi, B. Lngstroem, *J. Org. Chem*., **2002**, *67*, 3687 – 3692.
3. R.S. Bon, C. Hong, M.J Bouma, R.F Schmitz, F.J.J de Kanter, M. Lutz, A. Spek. V. Orru, *Org. Letts*., **2003**, *2*, 3759 – 3762.
4. K.K. Ncokazi, T.J. Egan, *Anal. Biochem*., **2005**, *338*, 306-319.
5. GraphPad Prism, 3.0, GraphPad Software Inc., 10855 Sorento Valley Rd . #203, San Diego, Ca 92121.